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1. Geriatrics 7:20 (Jan.) 1952.
2. South. M.J. 40:414 (May) 1947.

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THE EFFECTS OF CHORIONIC GONADOTROPIN IN ADULT MEN: INCREASED ESTROGEN AND 17-KETOSTEROID EXCRETION, GYNECOMASTIA, LEYDIG CELL STIMULATION AND SEMINIFEROUS TUBULE DAMAGE*

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CHORIONIC gonadotropin is known to stimulate growth and endocrine function of the immature human testis. When administered to cryptorchid boys, it can produce enlargement and descent of the testis and, with sufficient amounts, sexual development. In hypogonadotropic eunuchoids, it produces differentiation and growth of Leydig cells and secretion of androgen (1). Little information is available, however, regarding the effects of chorionic gonadotropin on the mature human testis.

The purpose of this study has been to determine the effects of chorionic gonadotropin on the structure and function of the adult human testis.

Received for publication February 11, 1952.

* The patients who form the basis of this report were studied at the Endocrine Clinic and Medical Wards of the City of Detroit Receiving Hospital.

** Department of Medicine, Wayne University College of Medicine. Supported in part by a grant to Dr. Maddock from the Schering Corporation, through the courtesy of Dr. E. S. Henderson.

† Department of Anatomy, State University of Iowa College of Medicine. Supported in part by a research grant to Dr. Nelson from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service (No. RG 1778).

TABLE 1. PATIENTS; CLINICAL AND LABORATORY FINDINGS

Patient	Age (yrs.)	Presenting problem	Pertinent physical findings	TESTES (before therapy)			Urinary gonadotropins*		
				Gross	Microscopic		Number of rats	Uterine stimulation	Ovarian weight (mg.)
					Leydig cells	Seminiferous tubules			
E.H.	35	Impotence	Normal adult male sexual development	Right—normal adult size; left—atrophic, half normal adult size	Right—moderately reduced in number; left—only occasional cells seen	Right—reduced rate of spermatogenesis; left—all tubules completely hyalinized	6	+	52.0
S.R.	33	Sterility	Normal adult male sexual development	Normal adult size	Slightly less than average in number	Spermatogenesis in progress bilaterally	5	+	62.0
R.R.	47	Impotence	Normal adult male sexual development	Normal adult size	Moderately reduced in number	Reduced rate of spermatogenesis	4	+	24.4
W.S.	67	Impotence	Normal adult male sexual development	Normal adult size	Markedly reduced in number	Active spermatogenesis in 60% of tubules; no spermatogenesis and peritubular fibrosis in 40%	5	+	18.3
O.F.	41	Impotence	Normal adult male sexual development	Normal adult size	Moderately reduced in number	Spermatogenesis arrested at primary spermatocyte stage	3	+	20.9
E.G.	45	Bilateral traumatic castration	Normal adult male sexual development	Absent	—	—	4	+	87.2
M.A.	54	Hypopituitarism	Absent body, axillary and pubic hair; penis normal size	Soft, half normal adult size	Absent; no recognizable precursors	Completely hyalinized	4	−	11.5
H.D.	58	Hypopituitarism	Absent body, axillary and pubic hair; penis normal size	Soft, half normal adult size	Absent; no recognizable precursors	Completely hyalinized	3	−	11.9
W.T.	50	Addison's disease	Sparse axillary and pubic hair, absent body hair; "Addisonian" pigmentation	Normal adult size	Normal numbers, many vacuolated	Active spermatogenesis in 40% of tubules; disorganized spermatogenesis and tubular fibrosis in 60%	4	+	27.9
						Uninjected control rats	23	−	11.9

* Each rat received the ultrafilter concentrate of a 12-hour urine specimen; the exception was Patient E.G., in whose case 3-hour aliquots were used. A 12-hour concentrate of normal male urine produces uterine stimulation and ovaries weighing from 10 to 40 mg. (16).

During the course of the investigation, an increase in estrogen excretion was regularly observed when the hormone was administered to men having functioning testes. The source of the estrogen, therefore, became one of the major problems of the study.

Previous investigations have indicated that the testis is a major source of estrogen in men: castration results in a drop in urinary estrogen level (2), eunuchs and eunuchoids excrete less than normal amounts of estrogens (3, 4), and estrogen has been recovered from human testes (5). Whether this estrogen arises from Leydig, Sertoli, or germinal cells, however, has remained largely in the realm of speculation, although some conflicting information on this problem has been obtained from studies of testicular tumors and human hermaphrodites. Huggins and Moulder (6) observed feminization of a male dog having a testicular neoplasm, which they diagnosed as a Sertoli cell tumor, and concluded that the Sertoli cells produce estrogen; Berthrong, Goodwin and Scott (7) reached the same conclusion from the study of a similar dog tumor. Teilum (8), studying human testicular and ovarian tumors, and Witschi and Mengert (9), studying the gonads of an hermaphrodite, also concluded that the Sertoli cells produced estrogen. On the other hand, Hunt and Budd (10), who observed gynecomastia in a man with a Leydig cell tumor, and Nelson (11), who studied the gonads of 5 "feminine" pseudohermaphrodites, presented evidence that the Leydig cells were the source of estrogen in these individuals.

It is felt that the present investigation, in addition to furnishing information regarding the effects of chorionic gonadotropin on the adult testis, offers a new approach to the problem of the origin of estrogen in men. To determine the respective roles of the testis and adrenal cortex in producing the changes that were observed, the hormone was administered to adult men with and without functioning testes and to a man having Addison's disease. Changes in estrogen and 17-ketosteroid excretion, signs, symptoms, and numbers of seminal fluid sperm have been correlated with alterations in testicular morphology occurring during the administration of chorionic gonadotropin.

METHODS

Patients and therapy

Chorionic gonadotropin was administered to 5 adult men having functioning testes (Patients E. H. to O. F., Table 1). The presenting complaint was decreased potency in 4 and sterility in 1. The men, ranging in age from 33 to 67 years, had normal adult sexual development, and none had climacteric symptoms or complete impotence; testicular biopsy specimens obtained before therapy revealed the presence of Leydig cells and some degree of spermatogenesis in each. Urinary gonadotropin titers were within

normal limits in 3 patients and moderately elevated in 2 patients. Each patient received 5,000 units of chorionic gonadotropin[1] intramuscularly three times weekly for forty-six to sixty-five days (Tables A to E, appendix).

The hormone was also administered to 3 men without functioning testes (Patients E. G., M. A., and H. D., Table 1). Patient E. G., who had been traumatically castrated thirty-four days prior to therapy, but was otherwise a normal male, 45 years old, received 5,000 units of chorionic gonadotropin three times weekly for forty-two days. Patients M. A. and H. D., ages 54 and 58, presented the clinical picture of hypopituitarism of ten and twenty years' duration, respectively. The adrenal cortices of both patients responded to ACTH, 20 milligrams every six hours for seven days; circulating eosinophils fell from 300 and 350 per cubic millimeter to 0; and 17-ketosteroids increased from less than 2, to 5.0 and 9.5 milligrams per twenty-four hours. The 2 men subsequently received 20 to 50 milligrams of ACTH daily throughout the period of study. The testes of both men were completely hyalinized; the seminiferous tubules could be identified only as hyaline ghosts; and Leydig cells and recognizable Leydig cell precursors were completely lacking. Urinary gonadotropin assays gave negative results in both patients. Each of the 2 men received 5,000 units of chorionic gonadotropin daily for sixteen days, then 1,000 units twice daily for thirty-two days.

Chorionic gonadotropin was also administered to a man lacking functioning adrenal cortices, who had functioning testes. Patient W. T. (Table 1), 50 years old, presented the classic picture of Addison's disease, having a history of weakness, weight loss and increasing pigmentation for five years. In confirmation of the diagnosis of primary adrenal cortical failure, no response was observed from the administration of ACTH, 20 milligrams every six hours for seven days; the number of eosinophils did not decrease, 17-ketosteroid excretion did not increase, and no subjective improvement was noted. Testicular biopsy revealed the presence of Leydig cells and some spermatogenic activity. Urinary gonadotropin excretion was moderately elevated and 17-ketosteroids were low, averaging 2.1 milligrams per twenty-four hours. Chorionic gonadotropin was administered in a dose of 500 units daily for eight days, followed by 5,000 units daily for two successive days.

---

[1] A. P. L., 20,000 units per vial in dry powder form, generously supplied by Ayerst, McKenna and Harrison, Ltd., was used throughout this study. Eight cc. of diluent, supplied by the manufacturer, was added to each vial prior to use. Thus, each 5,000-unit dose was contained in 2.0 cc. of solution. The solution was stored at 4°C.

Urinary estrogens

Urinary estrogens were determined by a modification of the methods of Heller and co-workers (12, 13). Since this method, which has given constant, reproducible results, has not been completely recorded elsewhere, it will be described here in detail:

Collection, hydrolysis, extraction and purification of urine: The 12-hour or 24-hour urine specimens are stored at 4° C. after the completion of each collection period. A 24-hour specimen of urine is acidified by the addition of 5 cc. of concentrated hydrochloric acid per 100 cc. of urine and the acidified urine boiled for twenty minutes under reflux condensers. The hydrolyzed urine is then extracted with chloroform for four hours in Wolfe-Hershberg continuous extractors, the chloroform extract evaporated to about 20 cc., and extracted once with 20 cc. of 5 per cent sodium bicarbonate. The sodium bicarbonate fraction, which has been assayed and found to contain no estrogen, is discarded. The chloroform extract is then extracted three times with 10-cc. portions of 10 per cent sodium hydroxide. The chloroform fraction, which contains nonphenolic steroids, is discarded. The sodium hydroxide fraction is acidified to litmus paper with concentrated hydrochloric acid and extracted three times with 10-cc. portions of ethyl ether. The ether is evaporated to between 5 and 10 cc., 1½ cc. of sesame oil is added, and the remaining ether evaporated. Finally, sufficient sesame oil is added to the purified urine extract so that the desired total amount for administration to each assay animal is contained in 3/8 cc.

Assay of urine extract: All materials to be assayed are administered subcutaneously in sesame oil (1/16 cc. twice daily for three days) to intact female Sprague-Dawley rats, 25 days old at the time of the first injection. In a few instances, urine extracts were administered to both intact and castrated rats, and entirely similar increases in uterine weight were produced. Autopsies are performed on the animals twenty-four hours after the last injection, and the uterus weighed before and after expressing any intraluminal fluid. Only the uterine weights after expression of intraluminal fluid are recorded in this presentation.

In assaying the urine extracts, the initial level tested is usually a 1½-hour or a 3-hour aliquot of urine. Then this initial amount is halved or doubled, using 2 rats per dose, until at least one level is found which produces an average uterine weight of 50 to 80 milligrams, which is the sensitive range of the method; usually this can be accomplished by the second assay. In the majority of determinations, at least 4, and usually 6 to 10, rats are used.

In order to express the results as absolute values, which might be easily compared, the increases in uterine weights obtained by administering urine extracts were compared with those produced by injection of known amounts of alpha[2]-estradiol benzoate. A representative assay is depicted in Figure 1, compared with the response produced by increasing doses of alpha-estradiol benzoate. The sensitive portion of the assay curve lay between 0.025 and 0.20 microgram of alpha-estradiol benzoate; 0.025 microgram produced no increase over the response of uninjected controls; 0.05

[2] The old terminology was used throughout this paper.

and 0.10 microgram doubled and trebled uterine weight, respectively; and 0.20 microgram produced almost the maximum response of four times the original uterine weight. A 3/8-hour aliquot of Patient S. R.'s urine, administered to 2 rats, produced uteri weighing 54 milligrams, a response similar to that obtained with 0.5 microgram of alpha-estradiol benzoate; a 3/4-hour and a 1½-hour aliquot approximated the responses obtained with 0.1 and 0.2 microgram of alpha-estradiol, respectively. By calculation,

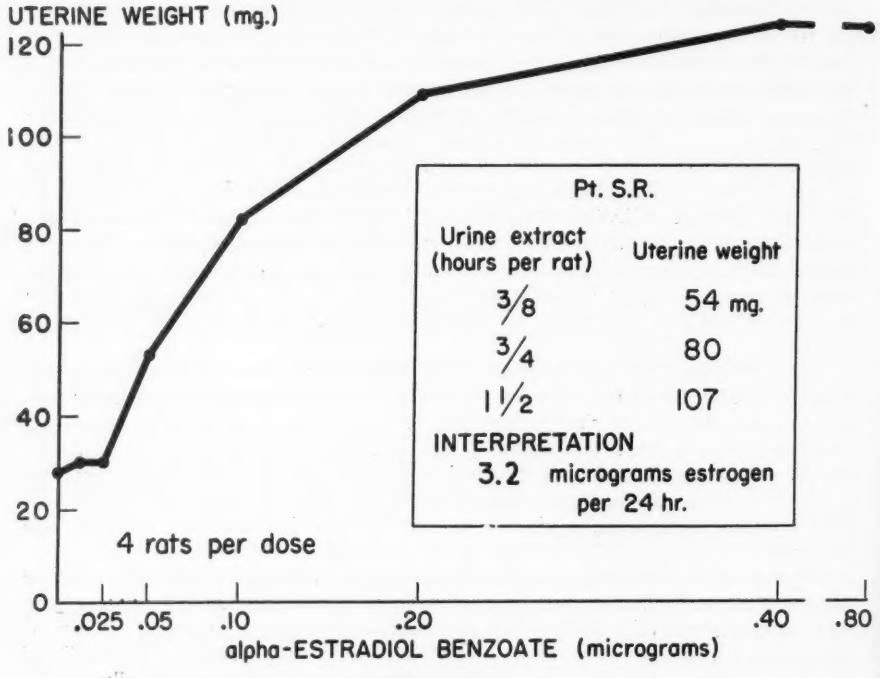


FIGURE 1

it is concluded that a 24-hour urine collection of Patient S. R. contained the equivalent of 3.2 micrograms of alpha-estradiol benzoate.

Urinary 17-ketosteroids

A modification of the method of Drekter and co-workers (14) was employed, using the Zimmermann color reaction. Since we used 12-hour overnight urine specimens and Pincus (15) has demonstrated that excretion of 17-ketosteroids is somewhat less during sleep than during the waking hours, our results are probably slightly lower than those which would have been obtained by using 24-hour urine collections.

Urinary gonadotropins

Assays were performed in immature female rats following concentration of the urine by ultrafiltration, as previously described (16).

Testicular biopsies

By incision of the scrotum and testes, specimens were obtained in all patients before therapy and repeated in 3 patients on the last day of therapy. A portion about 0.5 cm. in diameter was fixed in Bouin's solution, sectioned at 5 micra, and stained with Masson's trichrome stain, iron hematoxylin and a Mallory azan stain. A similar portion was fixed in 10 per cent neutral formalin for histochemical studies, including Sudan Black and Nile Blue stains, Schiff and Schultz reactions, and birefringence.

Sperm counts

Sperm were counted in a Neubauer counting chamber after diluting seminal fluid specimens with dilute aqueous methylene blue in a red or white cell pipette. Sperm were counted at intervals of from one to three weeks before, during, and after therapy in 3 of the patients.

RESULTS

Urinary estrogen levels: In the 5 patients having some testicular function, pretreatment urinary estrogen levels tended to be lower than normal, varying from 0.3 to 0.8 microgram per twenty-four hours (Figs. 2–6, and Tables A to E, appendix), compared with 0.6 to 1.2 micrograms per twenty-four hours for 17 normal men studied in this laboratory. (Results are depicted graphically in the figures; the data from which estrogen levels were computed is tabulated in the appendix, Tables A to I.) Estrogen levels were elevated in the first assay performed after initiating treatment, the time varying from the fourth to the twenty-second day of therapy. The increase in estrogen excretion was maintained throughout therapy, reaching a maximum of from five to sixteen times pretreatment levels. Estrogen excretion had dropped to pretreatment values or less in patients E. H., W. S., and R. R. seven to fourteen days after stopping therapy. In patient S. R., after cessation of therapy, estrogen excretion was still elevated on the ninth day, but fell to below pretreatment values the thirty-second day. Post-treatment studies were not obtained on patient O. F.

In the 3 men lacking functioning testes, no increase was found in urinary estrogens during chorionic gonadotropin therapy. In the castrated patient, E. G. (Fig. 7; and Table F, appendix), an initial high urinary estrogen level of 6.4 micrograms per twenty-four hours was encountered before therapy. An occasional, single, high urinary estrogen level following

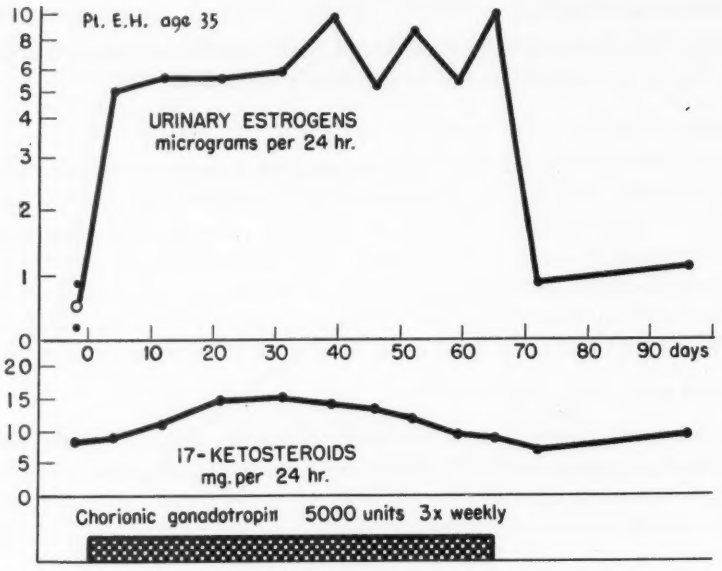
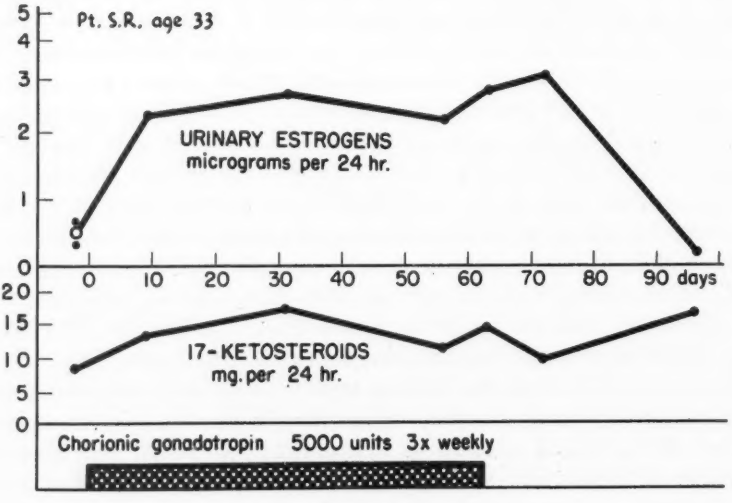


FIGURE 2



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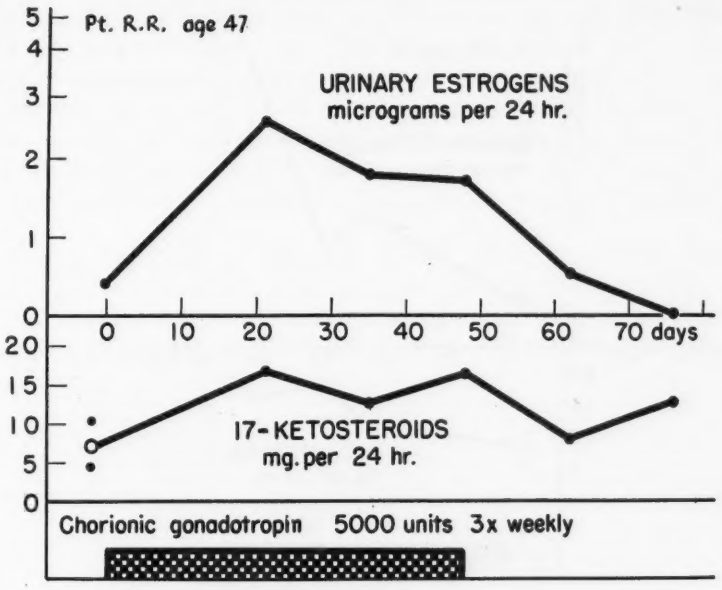

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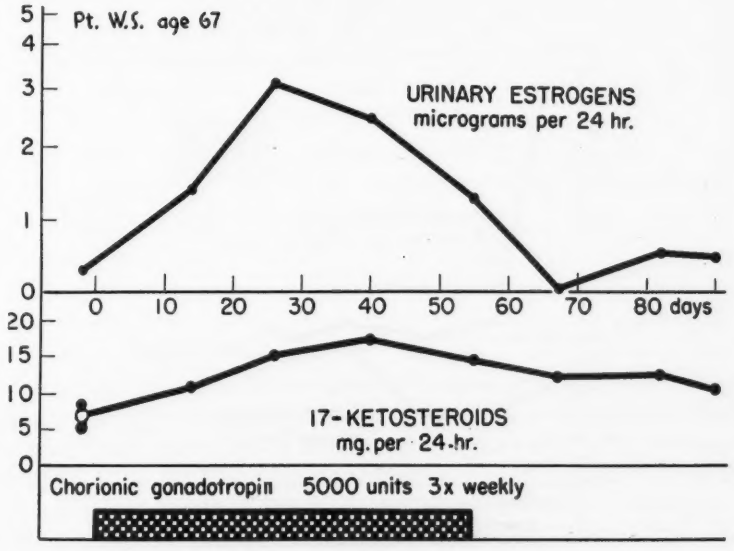

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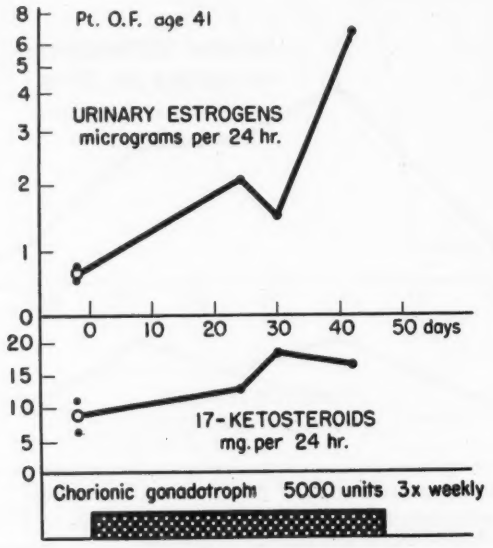

FIGURE 6

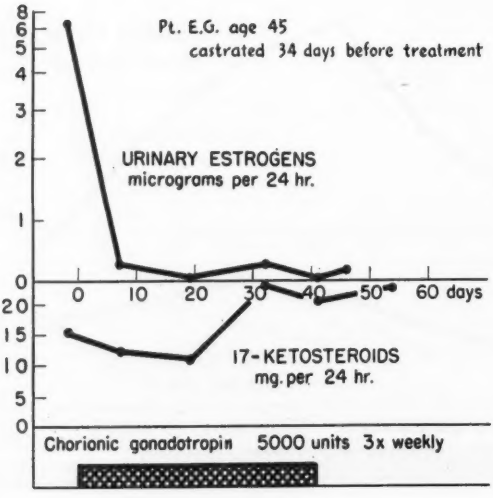

FIGURE 7

castration, similar to that encountered in our patient, has also been observed by Scott and Vermeulen (2). During and after forty-two days of chorionic gonadotropin therapy, however, estrogen excretion remained at very low levels, *i.e.*, from 0 to 0.2 micrograms per twenty-four hours. In the 2 patients with completely hyalinized testes, estrogen excretion remained at less than 0.2 microgram per twenty-four hours before, during, and after forty-seven days of chorionic gonadotropin therapy (Tables G and H, appendix).

In Patient W. T., with Addison's disease, whereas 500 units daily of chorionic gonadotropin had no effect, 5,000 units daily for two days produced a threefold increase in urinary estrogen excretion within the first twenty-four hours (Fig. 8; and Table I, appendix). Three days following therapy, estrogen excretion fell to almost pretreatment levels.

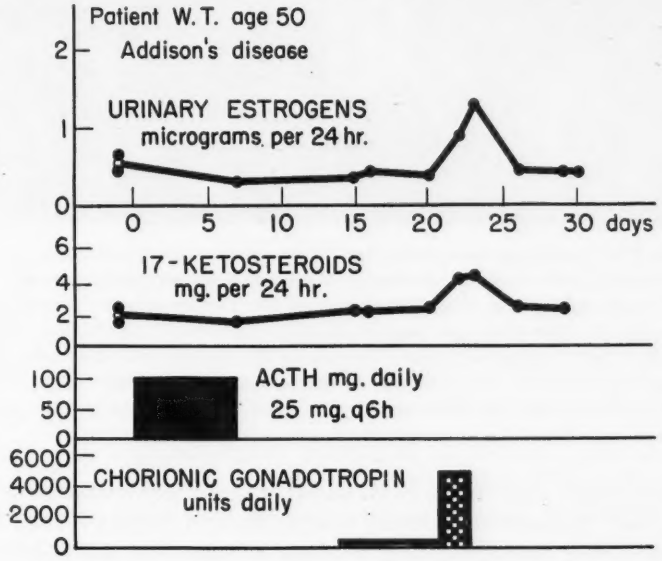

FIGURE 8

Urinary 17-ketosteroids: In contrast to the marked increase that occurred in urinary estrogen excretion, urinary 17-ketosteroids increased only to about two times initial levels in each of the patients having functioning testes (Figs. 2 to 6). Furthermore, variations in 17-ketosteroid excretion bore no constant relationship to variations in estrogen excretion. In Patient E. H. (Fig. 2), estrogen excretion had increased ten times over pretreatment levels four days after initiating therapy, whereas 17-ketosteroids

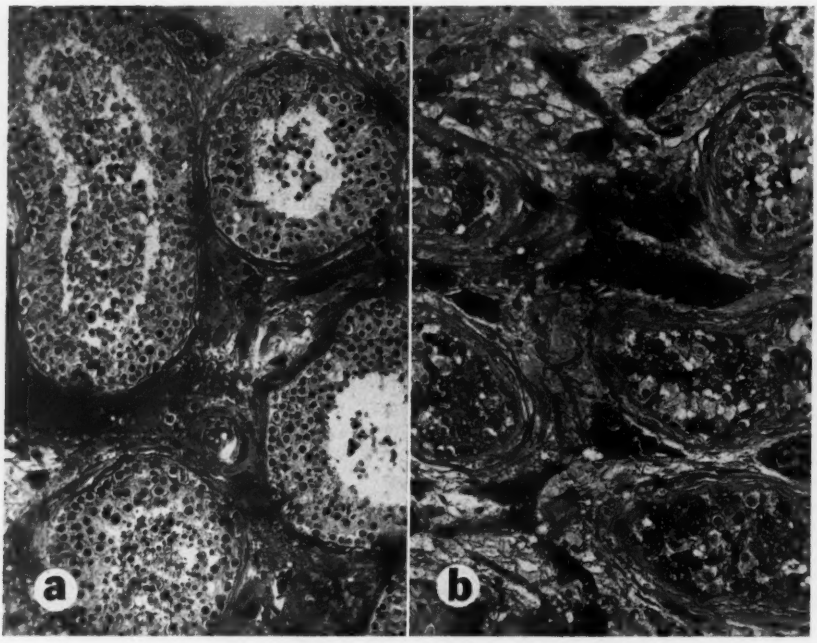

FIG. 9. Photomicrographs of testicular biopsy specimens of patient E. H. (×135). *a*. Before therapy: Seminiferous tubules are of normal size, spermatogenesis is in progress and only slight peritubular fibrosis is present. A few Leydig cells are seen in the interstitial spaces. *b*. After sixty-five days of chorionic gonadotropin therapy, 5,000 units three times weekly: Note the marked increase in the number of Leydig cells, which are small and dark-staining. Seminiferous tubule damage is apparent. The tubules are reduced in size, lumens are obliterated by sloughed, immature germ cells, peritubular fibrosis is increased and the basement membranes are hyalinized.

had not changed. In Patients S. R. and R. R., estrogen excretion decreased to less then pretreatment levels thirty-two and twenty-eight days, respectively, after therapy ended, whereas at this time the 17-ketosteroid levels approximated those found during therapy. The maximum increase in 17-ketosteroid excretion coincided with the maximum increase in estrogen excretion only in Patient R. R. (Fig. 5).

In the castrated patient, urinary 17-ketosteroids did not increase during the first three weeks of chorionic gonadotropin therapy, but subsequently rose to approximately one and one-half times pretreatment values and remained at that level after therapy ended (Fig. 7). That this increase is probably unrelated to therapy is indicated by the observation of Scott and Vermeulen (2), that 17-ketosteroid excretion is usually increased during the first few months following bilateral orchidectomy. In 2 patients with

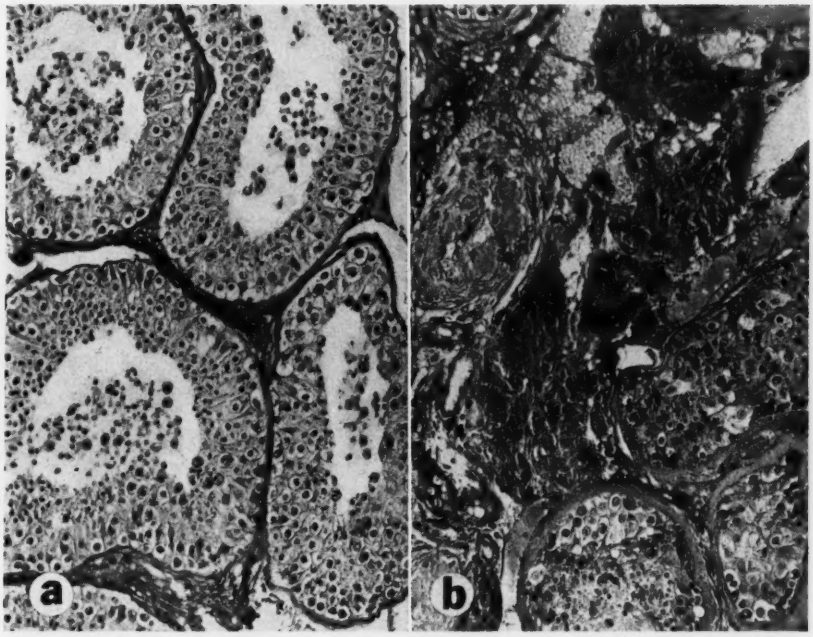

FIG. 10. Photomicrographs of testicular biopsy specimens of patient R. R. (×135). *a.* Before therapy: Seminiferous tubules are of normal size. Rate of spermatogenesis is reduced. (Leydig cells, which are not seen in this section, were reduced in number.) *b.* After forty-eight days of chorionic gonadotropin therapy, 5,000 units three times weekly: Leydig cells are increased in number. Marked seminiferous tubule damage is present, including hyalinization of basement membranes.

hypopituitarism and completely hyalinized testes, who were receiving ACTH before, during, and after the period of study, no further increases in 17-ketosteroid excretion were noted when chorionic gonadotropin was given.

In the patient with Addison's disease, no increase in 17-ketosteroid excretion occurred with daily administration of 500 units of chorionic gonadotropin for six days, but with 5,000 units for two successive days, ketosteroid excretion doubled (Fig. 8).

Testicular biopsies: Photomicrographs of the testes of each of the 3 patients from whom biopsy specimens were obtained both before and after therapy are presented in Figures 9 to 11.

In each patient pretreatment biopsies revealed some Leydig cells, although in reduced numbers. Less than the normal numbers of mesenchymal cells appeared to be undergoing transformation to mature Leydig

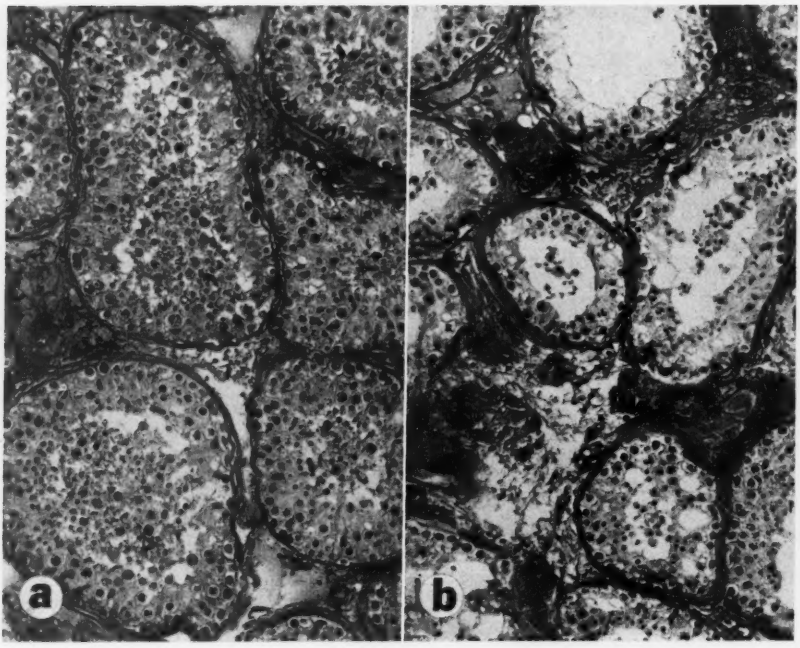

FIG. 11. Photomicrographs of testicular biopsy specimens of patient W. S. (×135). *a*. Before therapy: Leydig cells are markedly reduced in number. Seminiferous tubules are of normal size, and spermatogenesis is in progress. *b*. After fifty-six days of chorionic gonadotropin therapy, 5,000 units three times weekly: Abundant Leydig cells are now present. Seminiferous tubules are moderately reduced in size, spermatogenesis is reduced in rate and there is a slight increase in peritubular fibrosis.

cells, since the usual distribution of small and medium, dark-staining cells and large, poorly granulated vacuolated cells seen in normal testes was lacking. Biopsy specimens obtained at the conclusion of therapy revealed in each patient a substantial increase in the number of Leydig cells, which were, in general, small, dark-staining and heavily granulated. In Patient E. H. the number of Leydig cells was definitely greater than that seen in normal testes, whereas in Patients W. S. and R. R. the number did not exceed that seen occasionally, but not commonly, in normal testes.

Cytochemical studies demonstrated that the number of lipid droplets in the interstitial cells increased after treatment with chorionic gonadotropin because of the increased number of cells which contained them. Furthermore, these droplets tended to be appreciably smaller, a characteristic probably associated with an increased rate of hormone production.

The Schultz reaction, regarded as indicative of cholesterol and its esters, was regularly less evident after treatment. Since the cells which show this reaction most prominently are the large, vacuolated elements, it seems likely that the Schultz reaction is not necessarily associated with the most active interstitial cells. No changes in birefringence could be detected.

Before therapy, seminiferous tubules were of good size, although some degree of peritubular fibrosis and/or hyalinization of basement membranes was present in each patient. Spermatogenesis was in progress, although not to an optimum degree in any patient. At the conclusion of therapy, seminiferous tubules showed regressive changes in each patient, consisting of decrease in size of tubules, increase in peritubular fibrosis, basement membrane hyalinization, necrosis of germinal cells, and cessation of spermatogenesis. In none of the patients was any alteration in the number or appearance of Sertoli cells seen.

The cytochemical procedures failed to show spectacular changes in the tubules. Prior to treatment, the tubular lipids showed the increase usually associated with disturbed spermatogenesis (17, 18) and the reduction in spermatogenic activity following treatment was accompanied by only a moderate increase in lipids. Following treatment with chorionic gonadotropin, the lipid droplets in the Sertoli cells were slightly larger, a characteristic that is regarded as reflecting the disturbed trophic function of these cells.

Number of sperm: In Patients S. R. and R. R., the number of sperm fell to almost zero during therapy (Figs. 12 and 13). In Patient E. H., the number of sperm increased during the last two weeks of therapy, then fell to zero within one month after stopping therapy (Fig. 14). In Patients S. R. and E. H., the number of sperm subsequently increased to pretreatment levels or higher two months after the end of treatment.[3] The sperm count remained lower than pretreatment values in Patient R. R. up to eight months after cessation of therapy.

Symptoms and signs: During therapy, 2 of the 4 patients who had initially complained of decreased potency, observed an increase in the number of erections and in the frequency of intercourse. The patient who had complained of sterility noticed approximately twice the number of erections and frequency of intercourse, although he had not been aware of decreased sexual habits prior to therapy. Specific inquiries revealed no subjective changes, such as improved strength, endurance, or feeling of well being.

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[3] Patient E. H. received 5,000 units of pregnant mare serum five times weekly for five weeks, beginning five weeks after stopping chorionic gonadotropin therapy (Fig. 14).

During the second month of therapy, bilateral gynecomastia developed in Patients E. H. and S. R. In each it consisted of bilateral, firm, subareolar plaques 4 to 5 centimeters in diameter, associated with hyper-

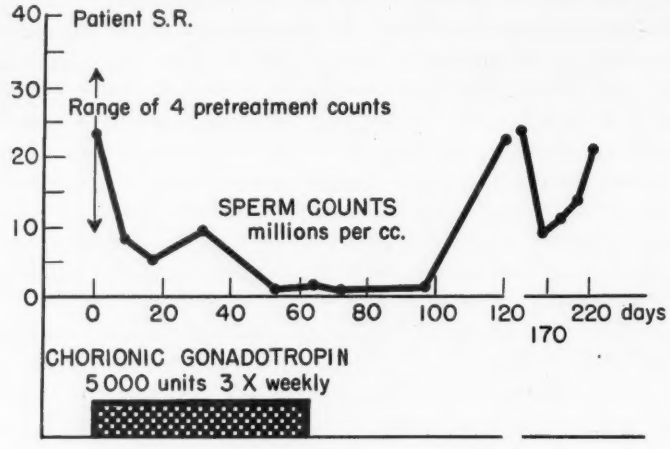

FIGURE 12

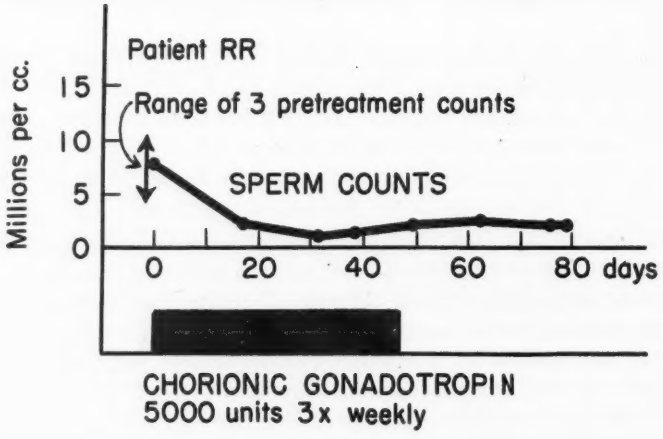

FIGURE 13

sensitivity of the nipples and areolae, similar to that produced by stilbestrol. The gynecomastia subsequently regressed one and three months, respectively, after the conclusion of therapy.

Although the 2 men with nonfunctioning testes responded to ACTH

therapy with symptomatic improvement in strength and mental outlook, impotence was unaltered either by chorionic gonadotropin or ACTH therapy; however, potency was restored in both patients when testosterone propionate was subsequently administered. The climacteric symptoms that the castrated patient experienced were unchanged by chorionic gonadotropin treatment, but were later abolished when testosterone propionate was administered. No subjective changes were noticed by the patient with Addison's disease during chorionic gonadotropin therapy.

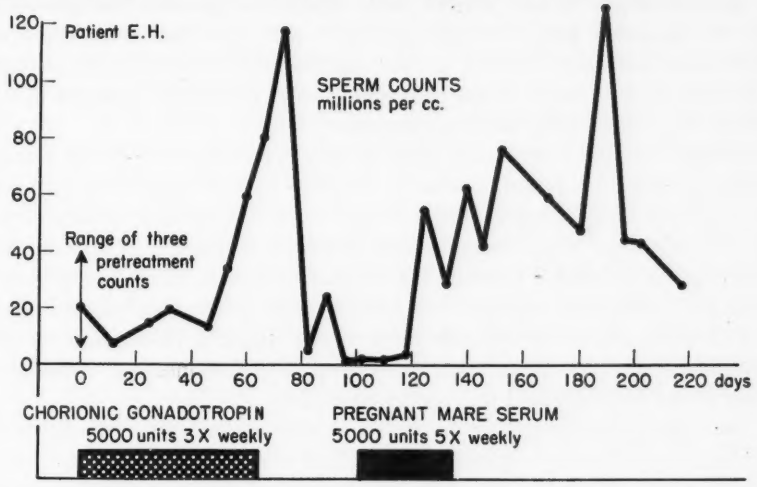

FIGURE 14

DISCUSSION

An increase in the urinary estrogen level appears to be a constant response to the administration of adequate amounts of chorionic gonadotropin in men having functioning testes, since each patient who possessed testes showed this response, whereas each patient who lacked testes did not.

The authors are unaware of any previous demonstration of the ability of chorionic gonadotropin to increase estrogen production in men. To our knowledge, the only investigation of this possibility was made by Kenyon *et al.* (19), who measured estrogen production before and during the daily administration of 1,500 units of chorionic gonadotropin to a boy 13 years old; although a positive nitrogen balance was induced, they were unable to demonstrate any increase in estrogen excretion. That the explanation may be inadequate dosage is suggested by the lack of increased estrogen excretion in Patient W. T. while he was receiving 500 units daily, whereas there was a definite increase while he was receiving 5,000 units daily.

Origin of the elevated levels of estrogen: The results of previous investigations suggest the testis and adrenal cortex as the two most likely sources of estrogen in men. Since the administration of chorionic gonadotropin resulted in an increase in estrogen excretion in men with functioning testes and none in men who lacked functioning testes, it would appear reasonable to assume that the testis and not the adrenal cortex was the source of the elevated levels of estrogen. Further evidence excluding the adrenal cortex was the increased excretion of estrogen during administration of the gonadotropin in the patient with Addison's disease. The possibility that the chorionic gonadotropin may have contained estrogens was eliminated, since administration of as much as 500 units to castrated rats failed to produce any increase in uterine weight, and estrogen excretion did not increase in men lacking functioning testes.

Having concluded that the testis is the site of the increased estrogen levels, the question remains whether Leydig, Sertoli, or germinal cells are the source of this estrogen. The association of increased numbers of Leydig cells together with histochemical changes indicative of enhanced secretion in each of the 3 patients from whom repeat testicular biopsy specimens were obtained suggests the Leydig cells as the most likely source. Furthermore, these Leydig cells were of the small, dark-staining, heavily granulated type which has been associated with the onset of secretory activity in the bull (20) and in puberal boys (21).

The regressive changes encountered in the seminiferous tubules at the conclusion of therapy suggest that neither Sertoli nor germinal cells are the source of estrogen. The accumulation of lipid within Sertoli and germinal cells, found in the histochemical studies of the biopsy specimens, is characteristic of defective spermatogenesis (17, 18) and probably represents a lack of utilization of nutrients by the germinal cells. Thus, the changes in tubular lipid observed in these patients are considered to be related to germinal cell degeneration rather than to hormone production. Further evidence that the cells within the seminiferous tubules are not the source of the estrogen is afforded by the presence of peritubular fibrosis and hyalinization of the basement membrane. It seems unlikely that the intratubular cells would be able to produce or deliver increased amounts of hormone to the circulation with a barrier of fibrous and hyaline tissue interposed between them and the capillaries. It is concluded, therefore, that the Leydig cells, rather than the Sertoli or germinal cells, are the source of the increased levels of estrogen observed in these studies.

Since administration of testosterone propionate results in an increased urinary excretion of both 17-ketosteroids and estrogens (22–25), the question remains whether the Leydig cells secrete estrogen directly or whether the estrogen is a degradation product of androgen secreted by Leydig cells

and then converted elsewhere to estrogen. The latter explanation seems unlikely, since a quantitative analysis of the data shows that estrogens increased to from five to sixteen times pretreatment levels during chorionic gonadotropin therapy, while there was only a twofold increase in 17-ketosteroids. Furthermore, variations in estrogen excretion bore no constant relationship to variations in 17-ketosteroid excretion. Although 17-ketosteroids are not synonymous with androgens, it has been shown that 17-ketosteroid determinations usually agree closely with the biologic assays for androgens (26). We, therefore, favor the concept that the Leydig cells secrete both estrogen and androgen, the two having no obligatory relationship to each other, as they would have if estrogens were derived solely from the metabolic conversion of androgen. This concept is in agreement with the observations of Rakoff (27), who demonstrated that in stallions the estrogen content of spermatic vein blood is about twenty times greater than that of other venous blood.

Mechanism of seminiferous tubule damage: The regressive changes occurring in the seminiferous tubules during administration of chorionic gonadotropin reflect the decreased numbers of sperm in the seminal fluid. These regressive changes may be caused by 1) direct depression of seminiferous tubules by chorionic gonadotropin, 2) direct depression of spermatogenesis by the increased endogenous androgen and/or estrogen, or 3) depression of pituitary FSH secretion by the increased estrogen and/or androgen. The first possibility seems unlikely, as Heller and Nelson (1) have demonstrated that pituitary FSH stimulates spermatogenesis in hypogonadotropic eunuchoids who are also receiving chorionic gonadotropin. The second possibility seems unlikely, since Moore (28) has shown that concomitant administration of gonadotropins will prevent the damaging effects of estrogens on the testis of the rat, and testosterone pellets implanted in the testes of hypophysectomized rats (29) and monkeys (30) maintain spermatogenesis. The third mechanism seems the most plausible, since the histologic changes encountered are similar to changes found when previously functioning seminiferous tubules are deprived of pituitary FSH stimulation by the following procedures: administration of large doses of androgens (31), the presence of antihormones effective against human pituitary FSH (32, 33), and destructive lesions of the anterior pituitary (34, 35). Direct evidence for inhibition of pituitary gonadotropin secretion was not obtained in this study, since it was not feasible to determine pituitary gonadotropin excretion when the patients were receiving chorionic gonadotropin.

That each patient with functioning testes responded to chorionic gonadotropin therapy demonstrates that adult human Leydig cells are capable of being stimulated, regardless of the patient's age (33 to 67 years) or the

level of endogenous gonadotropin excretion (normal to moderately elevated). Furthermore, the response occurs promptly within twenty-four hours after a single injection of 5,000 units. These findings may have practical applications, in view of the report of Landau *et al.* (36) that administration of chorionic gonadotropin to young adult men induced a positive nitrogen balance along with a rise in 17-ketosteroid excretion.

The occurrence of gynecomastia in 2 of the patients is believed to reflect the increased levels of circulating estrogens. There is no indication that chorionic gonadotropin acts directly on the breast, since gynecomastia was not observed when the hormone was administered to the 3 patients who lacked functioning testes. The gynecomastia occasionally observed in boys receiving chorionic gonadotropin for cryptorchidism and the breast enlargement observed by Geschickter and coworkers (37) in a male chimpanzee receiving the gonadotropin are also probably best explained on the basis of increased estrogen secretion.

Urinary estrogen excretion proved to be a much more sensitive and reliable index of the response of Leydig cells to stimulation with chorionic gonadotropin than did 17-ketosteroid excretion. The increases in the level of estrogen excretion were more marked, prompt, sustained and less variable than the increases in 17-ketosteroid excretion. One probable reason for this difference is that whereas in normal men slightly less than half the 17-ketosteroids are derived from the testis (the remainder from the adrenal cortex), most of urinary estrogens originate there (38).

The determination of changes in estrogen excretion produced by a standard dose of chorionic gonadotropin is suggested as a test of the functional capacity of the Leydig cells, similar to the test of Thorn, Forsham and co-workers (39), in which the change in 17-ketosteroid excretion produced by ACTH is used to measure "adrenal cortical reserve." It is the authors' opinion that the measurement of urinary estrogen excretion is one of the most valuable and least used tools for studying the secretory function of the human testis.

SUMMARY AND CONCLUSIONS

Urinary estrogen excretion increased to from five to sixteen times pretreatment levels in each of 5 adult men who received 5,000 units of chorionic gonadotropin three times weekly for periods of forty-seven to sixty-five days. Urinary 17-ketosteroid excretion increased to about twice pretreatment levels, but the changes were variable and bore no constant relation to changes in estrogen excretion. From 3 patients repeat testicular biopsy specimens were obtained at the conclusion of therapy; in each, Leydig cells were strikingly increased in numbers and showed histochemical changes indicative of enhanced secretory activity, while seminiferous

tubules were decreased in size and showed basement membrane hyalinization, peritubular fibrosis, cessation of active spermatogenesis, and necrosis and sloughing of germinal cells. The number of sperm in the seminal fluid decreased to almost zero. No change in the number of Sertoli cells and no histochemical changes indicative of stimulation were observed. In 2 patients gynecomastia developed during the second month of therapy. Estrogen excretion did not increase when chorionic gonadotropin was administered to 3 men who lacked functioning testes, but did increase when the hormone was administered to a man with Addison's disease.

The following conclusions, therefore, seem warranted:

1) The Leydig cells rather than Sertoli or germinal cells are the source of the estrogen elaborated by the human testis.

2) Adult, functioning Leydig cells respond to chorionic gonadotropin by secreting increased amounts of both androgen and estrogen.

3) Estrogen excretion is a more sensitive and reliable indicator of the response of Leydig cells to chorionic gonadotropin than is 17-ketosteroid excretion.

4) The seminiferous tubule damage (and concomitant decrease in the number of sperm in the seminal fluid) that occurs during the administration of chorionic gonadotropin is most likely due to suppression of pituitary FSH secretion by the increased levels of endogenous estrogen and androgen.

Acknowledgment

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APPENDIX

TABLE A. PATIENT E. H. URINARY ESTROGEN ASSAYS

Treatment: 5,000 units chorionic gonadotropin three times weekly for 65 days

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
0	1½	63	0.8
	3	80	
0	3	37	0.4
	3	44	
	3/16	42	
4	3/8	67	5.0
	3/4	101	
	1½	110	
	3/16	51	
12	3/8	71	5.6
	3	105	
	3/16	54	
21	3/8	67	5.6
	3	102	
	3/16	57	
31	3/8	67	5.9
	3/4	91	
	1½	115	
	3/32	51	
	3/16	70	
39	3/8	84	9.5
	3/4	116	
	1½	107	
	3/32	45	
52	3/16	72	9.0
	3/8	79	
59	3/16	50	5.4
	3/8	68	
	3/32	59	
65	3/16	61	9.9
	3/8	80	
	3/4	44	
72	1½	61	0.9
	3	74	
	3/4	51	
96	1½	59	1.1
	3	81	

TABLE B. PATIENT S. R. URINARY ESTROGEN ASSAYS

Treatment: 5,000 units chorionic gonadotropin three times weekly for 63 days

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
0	1½	51	0.7
	3	64	
0	1½	42	0.3
	3	45	
	⅜	45	
9	¾	60	2.3
	1½	95	
	3/16	42	
31	⅜	48	2.7
	¾	71	
56	⅜	40	2.2
	¾	67	
63	⅜	47	2.8
	¾	76	
	⅜	54	
72	¾	80	3.2
	1½	107	
	6	102	
	¾	33	
92	1½	32	<0.2
	6	20	

TABLE C. PATIENT R. R. URINARY ESTROGEN ASSAYS

Treatment: 5,000 units chorionic gonadotropin three times weekly for 48 days

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
0	3	46	0.4
	3	61	
20	3	130	2.6
	3	101	
	$\frac{3}{4}$	51	
34	1	66	1.9
	3	116	
	$\frac{3}{4}$	50	
48	1	67	1.7
	3	110	
61	3	63	0.5
75	3	40	<0.2

TABLE D. PATIENT W. S. URINARY ESTROGEN ASSAYS

Treatment: 5,000 units chorionic gonadotropin three times weekly for 56 days

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
0	3	50	0.3
	6	60	
0	3	53	0.3
	6	55	
	$\frac{3}{4}$	51	
14	$1\frac{1}{2}$	77	1.4
	3	98	
	$\frac{3}{4}$	55	
26	$\frac{3}{4}$	77	3.1
	3	117	
	$\frac{3}{4}$	57	
40	$1\frac{1}{2}$	106	2.4
	3	97	
55	$\frac{3}{4}$	49	1.3
	$1\frac{1}{2}$	61	
	$1\frac{1}{2}$	39	
67	3	39	<0.2
	6	43	
	$1\frac{1}{2}$	47	
82	3	67	0.6
	6	90	
	$1\frac{1}{2}$	43	
101	3	49	0.5
	6	90	

TABLE E. PATIENT O. F. URINARY ESTROGEN ASSAYS

Treatment: 5,000 units chorionic gonadotropin three times weekly for 46 days

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
0	1½	56	0.8
	3	69	
0	1½	45	0.6
	3	58	
24	¾	65	2.1
	1½	89	
30	1½	82	1.6
42	3/16	49	6.7
	¾	110	

TABLE F. PATIENT E. G. URINARY ESTROGEN ASSAYS

Treatment: 5,000 units chorionic gonadotropin three times weekly for 42 days

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
	3/16	55	
0	¾	74	6.4
	¾	110	
	3	143	
7	3	43	0.3
	6	60	
19	3	31	<0.2
	6	35	
32	3	37	0.2
	6	59	
41	6	39	<0.2
46	6	51	0.2

TABLE G. PATIENT M. A. URINARY ESTROGEN ASSAYS

Treatment: chorionic gonadotropin, 5,000 units daily for 16 days; then 1,000 units twice daily for 32 days

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
0	6	40	<0.2
8	6	35	<0.2
27	6	20	<0.2
41	6	36	<0.2
54	6	28	<0.2

TABLE H. PATIENT H. D. URINARY ESTROGEN ASSAYS

Treatment: chorionic gonadotropin 5,000 units daily for 16 days; then 1,000 units twice daily for 32 days

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
0	6	23	<0.2
0	6	44	<0.2
8	6	38	<0.2
27	6	27	<0.2
37	6	20	<0.2
47	6	24	<0.2
81	6	28	<0.2

TABLE I. PATIENT W. T. URINARY ESTROGEN ASSAYS

Treatment: 25 mg. ACTH every six hours for days 1 to 7; chorionic gonadotropin, 500 units daily, for days 14 to 21; then 5,000 units on days 22 and 23

No. of days after therapy initiated, urine collected	Hours urine extract per assay rat	Uterine weight (avge. of 2 assay rats), mg.	Estrogens per 24 hrs., as γ of α-estradiol benzoate
0	3	60	0.5
	6	93	
0	1½	48	0.6
	3	59	
7	3	53	0.3
	6	59	
15	3	52	0.4
	6	56	
16	3	57	0.4
	6	64	
20	3	56	0.4
	6	80	
22	1½	58	0.9
	3	87	
23	1½	83	1.4
	3	94	
26	3	58	0.5
	6	93	
29	3	56	0.5
	6	90	
30	1½	33	0.4
	3	54	

FURTHER STUDIES ON THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA WITH CORTISONE

III. THE CONTROL OF HYPERTENSION WITH CORTISONE, WITH A DISCUSSION OF VARIATIONS IN THE TYPE OF CONGENITAL ADRENAL HYPERPLASIA AND REPORT OF A CASE WITH PROBABLE DEFECT OF CARBOHYDRATE-REGULATING HORMONES*

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IT IS generally accepted that the adrenal cortex plays a role in the maintenance of blood pressure. Hypotension is a symptom of Addison's disease, and hypertension occurs in Cushing's syndrome. The administration of excessive amounts of desoxycorticosterone or of cortisone raises the blood pressure. Thorn and his associates (1) have treated patients with essential hypertension by bilateral complete adrenalectomy followed by substitution therapy with cortisone and desoxycorticosterone. There was no evidence in the cases treated that the hypertension was caused by excessive or abnormal adrenal hormones.

In spite of its frequency in Cushing's syndrome, hypertension is uncommon in the adrenogenital syndrome. It has been described in a number of cases of adrenocortical tumors of the virilizing type (2, 3). One male patient with congenital adrenal hyperplasia and hypertension who improved with cortisone therapy has been reported by Shepard and Clausen (4). In a previous paper (5) the association of these conditions was mentioned in 3 patients whom we shall describe now in more detail. In addition, studies will be reported suggesting that one of these patients had deficiency of the carbohydrate-regulating hormones of the adrenal. We shall take the opportunity to discuss the variations of symptomatology in cases of con-

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genital adrenal hyperplasia and the probability that there may be a number of different patterns of abnormal adrenal activity.

CASE REPORTS

J. S. (92456) was a female pseudohermaphrodite of 18½ years who, in addition to the usual signs of congenital adrenal hyperplasia, had persistent hypertension and marked pigmentation of Addisonian type. At various times during childhood, blood pressure readings from 125/65 to 150/90 were recorded. Six months prior to treatment with cortisone, hypertension varying from 170/115 to 210/150 was found. Slight cardiac

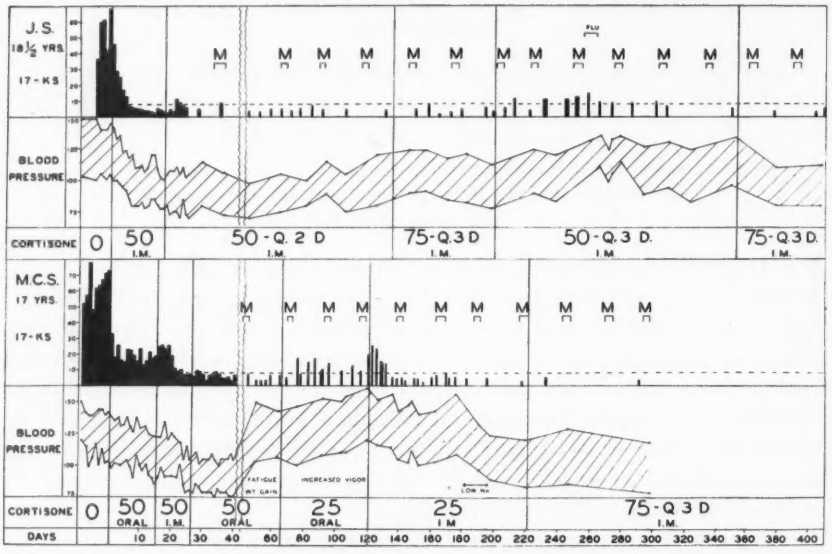

FIG. 1. Showing effect of cortisone therapy on the urinary 17-ketosteroids and the blood pressure in 2 sisters with female pseudohermaphrodism due to congenital adrenal hyperplasia. The blood pressure has remained normal after nineteen and fourteen months of treatment, respectively.

enlargement and slight sclerosis of the retinal arteries were noted. Renal function tests gave normal results and a test with benzodioxane elicited no response. Treatment by Dr. John E. Howard with a low salt diet caused no significant lowering of blood pressure, which at no time was below 150/100.

Continuous treatment with cortisone, given intramuscularly, was begun in June 1950. The decreased hirsutism, the feminization with breast development, changes in the vaginal smear and establishment of regular menstrual cycles with basal temperature fluctuations, which occurred with treatment, are described elsewhere (5). The effects on the urinary 17-ketosteroids and the blood pressure are shown in Figure 1. With 50 mg. of intramuscular cortisone given daily, the urinary 17-ketosteroids fell promptly from an average level of 53 mg. per day to an average of 4.5 mg. per day. The blood pressure fell in a manner almost parallel with the decline of ketosteroids, from levels between 140/100 and 150/105, to 100/76. When the daily dose of cortisone was decreased to 25 mg., the

suppression of the urinary 17-ketosteroids was maintained at averages of 5 to 6 mg. per day. The administration of 50 mg. of cortisone every other day was as effective as 25 mg. injected daily. During the first 200 days of treatment the blood pressure did not exceed 125/90 and on the 200th day it was 112/76. Between the 200th and 356th days of treatment the dose of cortisone was reduced to an average of 16.6 mg. per day (50 mg. every third day) and the urinary 17-ketosteroids increased to an average of 9.6 mg. per day, sometimes reaching as high as 15 mg. per day. During this period the blood pressure rose again to levels between 125/90 and 138/115. Subsequently an increase in the dose of cortisone to 75 mg. every third day resulted in suppression of the urinary 17-ketosteroids to a level of 3.8 mg. and a fall in the blood pressure to its previous lower value. OnNovember 10, 1951, it was 112/88. Cardiac enlargement and retinal sclerosis were no longer present.

There were no apparent factors contributing to the lowering of the blood pressure except the suppression of adrenal activity by cortisone. At the time treatment was begun, the patient was receiving the relatively low salt diet which she had taken for over six months. On leaving the hospital she resumed a normal diet without salt restriction. In the hospital she was not confined to bed and her activity was not restricted and included walks and shopping. On returning home she resumed the life of an active college girl.

M. C. S. (92457) was the 16¼-year-old sister of J. S. Their conditions were practically identical, including pigmentation and hypertension which had been present for a number of years. On November 4, 1950, treatment with cortisone was begun by the oral instead of the intramuscular route (Fig. 1). With 50 mg. of oral cortisone given daily in two or four divided doses, the urinary 17-ketosteroids, which averaged 62 mg. per day before treatment, were reduced only to an average of 17 mg. per day. During this period the blood pressure, which was 140–150 systolic and 100–120 diastolic before treatment, was not very markedly reduced. When treatment was changed from the oral to the intramuscular route, there was a sharp drop of the 17-ketosteroid excretion on the sixth day, to a level of 8 mg. per day. With this, the blood pressure fell abruptly to a level of 105/77. After eleven days of intramuscular cortisone, a dose of 50 mg. a day by mouth was tried again. For the next fourteen days the blood pressure remained depressed. After this, the patient's condition changed remarkably. The blood pressure rose to the previous level of 150/105. The patient complained of weakness and fatigability and said that she felt tired an hour after rising in the morning. She slept from ten to fifteen hours each day. Her weight increased 3 Kg. within four weeks, in spite of the fact that her appetite was not increased, and her face became somewhat round but not florid. She complained also of feeling cold. The serum cholesterol level showed no significant rise, being 222 mg. per cent; and there were no obvious signs of hypothyroidism. It was suspected that she might be receiving an excessive amount of cortisone and that she might be retaining sodium. The serum electrolyte levels, however, remained normal; Na 148 mEq./L., K 3.9 mEq./L., Cl 106 mEq./L. and CO_2 combining power 25.8 mEq./L. The glucose tolerance curve was also normal. During this time the average 17-ketosteroid output was 6 mg. per day. The dose of cortisone was reduced to 25 mg. by mouth daily. Within two weeks she was greatly improved. She no longer felt drowsy and fatigued, but recovered her previous energy and vigor. Her weight decreased 1 Kg. In spite of her feeling of general well-being, the blood pressure remained elevated. Following the reduction in the dose of cortisone, the output of 17-ketosteroids increased to an average of 13.4 mg. per day, with a range from 8 to 19 mg. per day. The increased secretion of androgen was manifested by some coarsening of the hair on her face and legs. It was believed now that the persistence of hypertension might be due to the fact that the daily dose of 25 mg. of

cortisone given orally was not sufficient to suppress adrenal secretion adequately. Treatment was changed to 25 mg. of cortisone by intramuscular injection. This decreased again the excretion of 17-ketosteroids to a level of 4.8 mg. per day. The blood pressure, however, did not fall after sixty days of this regimen and the patient's weight increased slightly. Finally a low sodium diet was instituted. Between April 29 and May 19, 1951, there was a progressive fall of the blood pressure to 124/88 and the weight decreased from 53 to 50.2 Kg. The patient complained bitterly of the salt restriction. She claimed that she felt tired and listless and was emotionally depressed. At times she thought she was going to faint. Neither hypoglycemia nor change of serum electrolyte levels was demonstrable. Since the strict regimen seemed to be psychologically harmful, the patient was advised to discontinue it but to restrict her salt intake to as little as necessary to make her food palatable. Within a few days she was happy and feeling better. The blood pressure has remained at normal levels and on December 28, 1951, was 122/86. The weight has remained stationary. The suppression of urinary 17-ketosteroids and diminished hirsutism have continued during treatment with 75 mg. of cortisone given intramuscularly every third day.

W. J. (A-68429) was a boy who was first studied at the age of 20 months when he was admitted to the hospital with hypertension and cardiac decompensation on February 3, 1949. His height-age was 4 years and his bone-age, 6 years. The penis and prostate were enlarged, Acne and a slight growth of pubic hair were present. The testes, however, were not appreciably enlarged for a boy of his age. The fact that the sexual precocity was of adrenal origin was confirmed by finding an output of 17-ketosteroids of 6 mg. per day.

The relation of the hypertension to the adrenal disorder aroused immediate interest. A benzodioxane test was made, with negative results. No adrenal tumor was demonstrated by pyelograms or aortic angiograms. After digitalization and improvement in the cardiac condition, on March 2, 1949, an exploratory laparotomy was performed; it revealed that both adrenals were greatly enlarged. The left, weighing 16 grams, was removed. The cortex was everywhere of a dark brownish color.

We are indebted to Dr. Stanton Eversole of the Department of Pathology for the following description of the microscopic appearance of the adrenal (Fig. 2):

"The glomerulosa zone is well demarcated from the rest of the cortex and is everywhere two or three times its normal width. The cells of this zone are larger than normal and have cytoplasm which stains faintly basophilic. The rest of the adrenal cortex is also hyperplastic and is composed of rather large cells without orderly arrangement, so that the distinction between the fascicular and reticular zones is not clearly defined. The adrenal medulla is not hyperplastic. In the cortex and medulla there are no hyalinized arterioles such as are found in some cases of essential hypertension."

The left testis was exposed at operation and a dark brown nodule, 4 mm. in diameter, was seen on the surface. The entire testis with the nodule was removed. Microscopic sections showed (Dr. Eversole) that "the nodule was composed of hyperplastic adrenal tissue identical with the less orderly portions of the adrenal cortex. The testis was composed of seminiferous tubules which were widely separated by very loose connective tissue. The Sertoli cells appeared inactive and small. There was no spermatogenesis. Compared to the testes of normal children, one gets the impression that the general development of the patient's testes was perhaps less than normal."

Postoperatively the patient was given adrenal cortical extract for five or six days, together with NaCl, glucose, digitalis, oxygen therapy and antibiotics. The postoperative course was not unusually severe. Later, various studies were made but did not yield

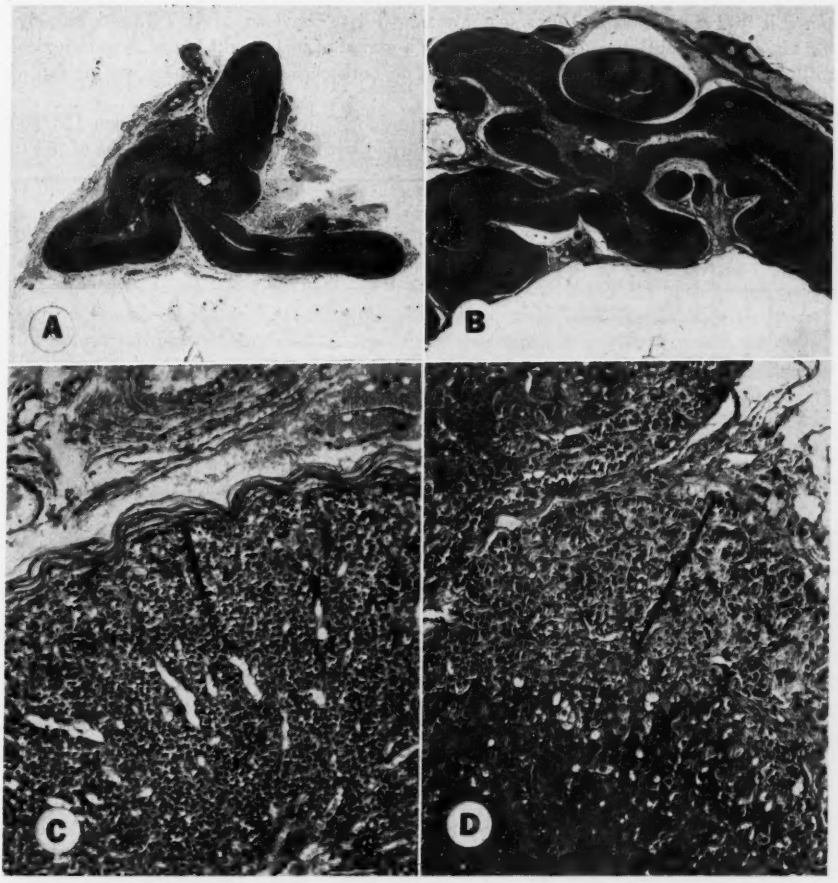

FIG. 2. A. Normal adrenal (×7). B. Adrenal of patient W. J., showing marked hyperplasia (×7). C. Normal adrenal (×150). Wide black line indicates width of zona glomerulosa. Note orderly arrangement of zona fasciculata. D. Adrenal of Patient W. J. (×150). Wide black line shows greatly increased width of zona glomerulosa. The rest of the cortex is composed of cells without orderly arrangement, resembling those of the zona reticularis. The zona fasciculata cannot be recognized. Only a portion of the greatly thickened cortex is shown in this section.

much information regarding the pathologic physiology of the adrenal and its relation to the hypertension. Repeated examinations failed to reveal any changes in the serum electrolytes or blood volume. The administration of desoxycorticosterone-glucoside, 1.5 mg. daily for two days, caused sodium retention without increasing the hypertension. The administration of estradiol dibenzoate, 0.1 mg. daily for fourteen days, had no effect on the blood pressure, although it caused slight breast enlargement. Likewise, two attempts to stimulate the adrenals with ACTH did not increase the hypertension, but it is

possible that actual stimulation was not obtained with the doses of ACTH used, namely, 4.5 mg. intramuscularly every six hours over a period of forty-eight hours. A paradoxical fall of urinary 17-ketosteroids was observed on both occasions and there was no fall of circulating eosinophils.

During the period up to January 1950, the blood pressure ranged between 155/100 and 168/110. The heart was moderately enlarged with cardio-thoracic indices of .55 to

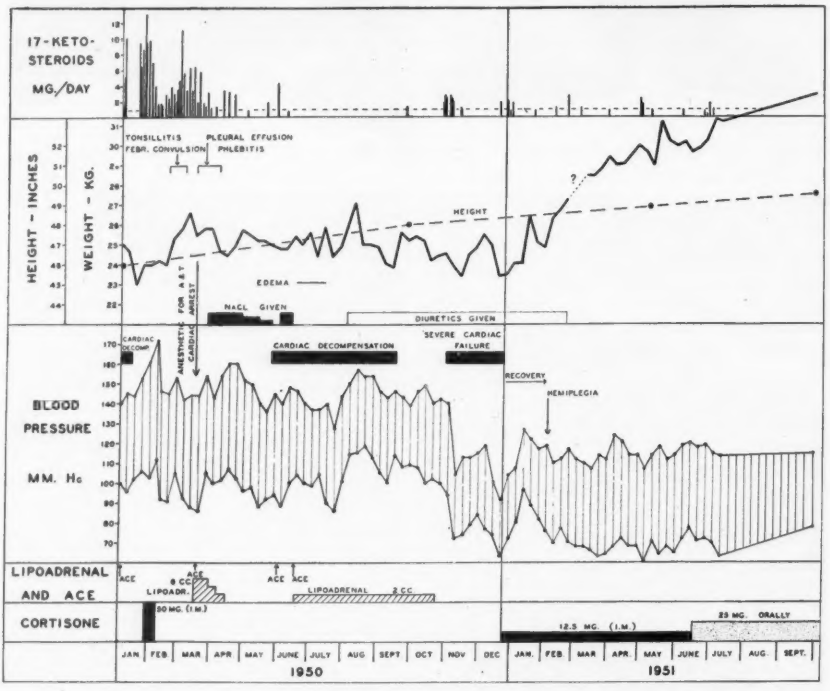

FIG. 3. Clinical course in the case of W. J., aged 3 years. During the first course of treatment with 50 mg. of intramuscular cortisone daily, the patient became excited and the blood pressure rose. The fall of the blood pressure in November and December 1950 was probably due to terminal cardiac failure. Treatment with 12.5 mg. of cortisone (i.m.) improved cardiac function and maintained the blood pressure at normotensive levels. The pressure has remained normal for twelve months under continuous therapy.

.60, measured on teleroentgenograms. At one period when digitalis therapy was neglected at home, cardiac decompensation recurred. In August 1949, the child was brought back to the hospital in hypoglycemic convulsions. Subsequently studies were made which suggested that there might be a deficiency of the carbohydrate-regulating hormones of the adrenal. These will be discussed later.

In October and November 1949, the patient was in a convalescent home without digitalis therapy. An upper respiratory infection developed and on January 5, 1950, he was readmitted to the hospital in a state of severe cardiac decompensation with fever, a

rapid thready pulse, rales, and an enlarged liver. With penicillin and digitalization he improved within two weeks. His course from this time on is shown in Figure 3. From January 30 to February 9, 1950, he was given cortisone in a dose of 50 mg. per day by intramuscular injection. The urinary 17-ketosteroids which were ranging from 6.6 to 13.3 mg. per day, with an average of 9.6 mg. per day, fell within five days to a level of 1.4 mg. per day. The patient became excited and manic and the blood pressure rose to a peak of 210/140. Cortisone was discontinued and the 17-ketosteroids rose to a level of 11 mg. per day by March 3.

The patient's condition deteriorated progressively during the next nine months. At the end of February he had a tonsillar infection and cervical adenitis, during which he had a convulsion. March 16 a tonsillectomy was planned. When the anesthetic was first started the patient's heart stopped. Thoracotomy was performed hastily and heart action restored by massage. During the next three weeks he was very ill with pleural effusions requiring thoracenteses, and phlebitis developed following intravenous drips. Several episodes of circulatory collapse aroused the suspicion that there might be adrenal insufficiency, although no changes in serum electrolyte levels were found. Accordingly, lipoadrenal extract was given between March 16 and April 11, with infusions of aqueous adrenal extract when he seemed worse. The addition of 3 grams of NaCl to the diet was begun and continued until June 21. As some edema developed in June, the salt was discontinued and diuretics were given. In spite of this, the belief that there might be a generalized adrenal insufficiency, or at least some deficiency of the glycogenetic hormones, led to the administration of 2 cc. of lipoadrenal extract daily from June 22 to October 19. In retrospect, it seems probable that the administration of salt together with adrenal hormones which might cause salt retention may have put an additional strain upon the heart. In November, following a respiratory infection, the patient's condition began to deteriorate more rapidly and the blood pressure, which until then had remained at levels between 140/100 and 150/110, fell progressively to 90/60. Throughout this period, in spite of constant digitalization, there were signs of cardiac failure such as cough, rales, puffiness of the face, and at times enlarged liver and albuminuria. The heart became progressively larger, the cardio-thoracic index increasing from .58 to .74.

On December 22, 1950, when death seemed imminent, cortisone therapy was tried again. This time a dose of 12.5 mg. of intramuscular cortisone was given daily, whereas previously the dose had been 50 mg. per day. There was rapid improvement in the cardiovascular condition. Within a few weeks the blood pressure rose from 92/63 to 127/97. However, the systolic pressure did not continue to rise but levelled off between 107 and 120, while the diastolic pressure dropped to between 60 and 72. The signs of cardiac failure disappeared and the general health improved remarkably. On February 4, 1951, a cerebrovascular accident occurred, resulting in a left-sided hemiplegia which has persisted. This was probably associated with the effect upon damaged cerebral arteries of the restoration of a normal cardiac output after prolonged impairment.

During the spring and summer the patient continued to improve. The blood pressure remained normal and the heart well compensated. Digitalis was discontinued in July and he was allowed to return home, where he led an active, unrestricted life. By August 1951 the cardio-thoracic index had decreased to .54, compared to .74 when cortisone therapy was begun in December 1950. On four occasions between July and November he had convulsive seizures followed by periods of unconsciousness. It was believed that these were the result of the cerebral vascular accident which had occurrred in February. On November 4, 1951, he was admitted again to the hospital immediately following a convulsive seizure. At this time the blood pressure was 150/110. Three days later it had

fallen to 100/60, and during the next month in the hospital it was usually at levels of 120–125 systolic and 80–85 diastolic. On December 29, 1951, three weeks after he had returned home, the pressure was 110/72. The urinary 17-ketosteroid excretion, which had ranged from 0.5 to 2.0 mg. per day with an average of 1.05 mg. per day between June and November, rose to 4 mg. per day on the day following the convulsion and then fluctuated between 1 mg. and 4 mg. per day during the next month.

In this case there is little direct evidence that the decrease of hypertension which

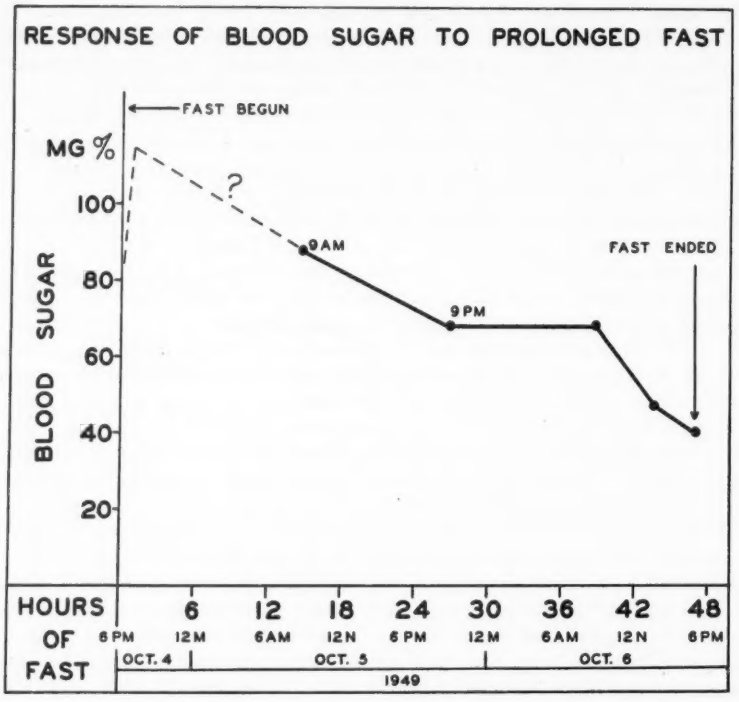

FIG. 4. Patient W. J. Blood sugar did not fall to hypoglycemic level until after thirty-nine hours of fasting.

followed cortisone therapy was due to suppression of adrenal activity, because there was no close correlation between the height of blood pressure and the level of 17-ketosteroid excretion, such as occurred in the case of J. S. Prior to the first short course of cortisone in February 1950, the urinary 17-ketosteroids had varied at different times from 1.5 to 17.0 mg. per day, being generally at low levels during periods of decompensation and poor health. After discontinuing cortisone, the output increased again to 11 mg. per day, but during the severe illnesses from March to June the output fluctuated from 0.8 to 6.0 mg. From June 22 to October 19, 1950, when the patient was receiving 2 cc. of lipoadrenal extract daily, the single determination of 17-ketosteroids which was made showed an output of 0.87 mg. per day. After lipoadrenal extract was discontinued the 17-ketosteroids varied between 1.2 and 2.8 mg. per day, but the patient's general condition was

very poor, and he was in a state of cardiac decompensation at the time. After cortisone was begun December 22, 1950, until November 1951, the average output of 17-ketosteroids was 1.3 mg. per day with a range of 0.5 to 2.7 mg. per day. Following the convulsion in November the blood pressure rose temporarily and the 17-ketosteroids fluctuated between 1 and 4 mg. per day.

The possibility that this patient had a deficiency of glycogenetic adrenal hormones, in addition to hypertension and excessive androgen secretion, was raised first by the oc-

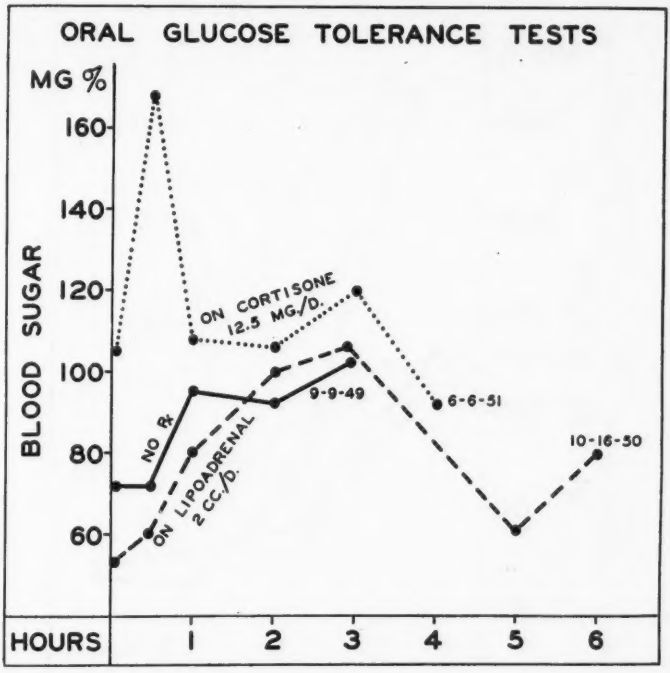

FIG. 5. Patient W. J. Low glucose tolerance curves before treatment with cortisone, and normal curve during treatment.

currence of a hypoglycemic episode on August 19, 1949. For two or three days previously he had been listless and had not eaten well. The day of the attack he ate nothing. At 5 P.M. he became semicomatose and generalized convulsive movements developed. On admission to the hospital his blood sugar was 18 mg. per cent and the spinal fluid sugar, 24 mg. per cent, An intravenous injection of 15 cc. of 50 per cent glucose was given and an intravenous drip of 5 per cent glucose with 10 cc. of aqueous adrenal extract was started. He was given also intramuscular injections of lipoadrenal extract. There was gradual recovery during the next two days.

At different times during the two and one-half years of observation various studies were made bearing on the patient's carbohydrate metabolism. No other hypoglycemic attacks have occurred. Although 92 blood sugar determinations were made at various times of the day, hypoglycemic levels were found on only two occasions, aside from the

attack described. One of these was two days after the left adrenalectomy. On October 4, 1949, an attempt was made to induce hypoglycemia by fasting. As shown in Figure 4 the blood sugar fell below 50 mg. per cent only after the patient fasted for thirty-nine

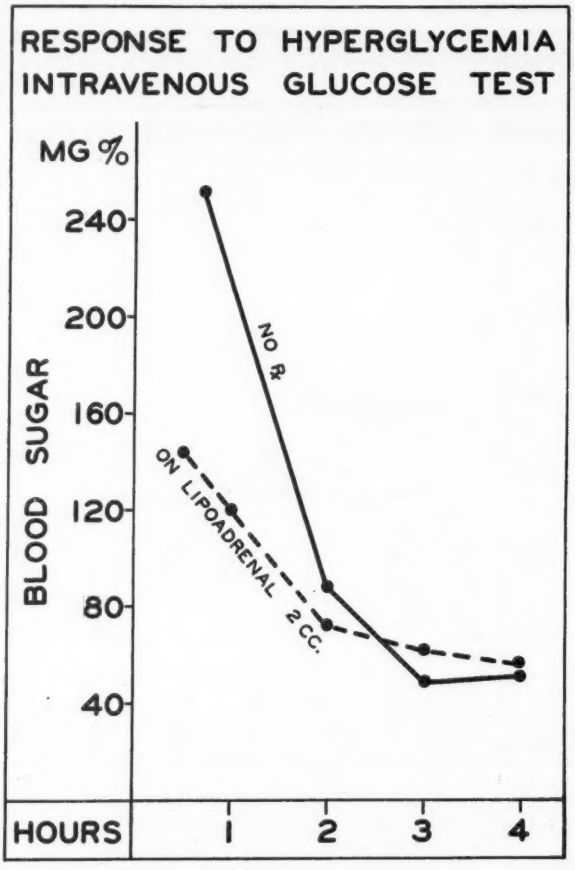

FIG. 6. Patient W. J. Before treatment with cortisone, hyperglycemia induced by intravenous glucose was followed by a fall of blood sugar to hypoglycemic levels. There was no rise to normal levels within four hours.

to forty-three hours. Figure 5 shows oral glucose tolerance curves. When the patient was untreated and when he was receiving 2 cc. of lipoadrenal extract daily, the curves were decidedly flat with a slow rise to peaks not exceeding 104 mg. per cent. This might have been due to slow absorption, possibly because of impaired circulation. Under cortisone therapy the curve became normal—but the patient's cardiac condition also was improved. It is possible that the diminished rise of blood sugar might have been due to the fact that the action of insulin was unopposed by adrenal hormones. Figure 6 shows the

"response to hyperglycemia" induced by the intravenous injection of glucose. In both tests the blood sugar concentration fell to hypoglycemic levels and failed to rise again after three or four hours. This may have been due to lack of adrenal hormones antagonistic to insulin. Figure 7 shows three tests to determine the patient's ability to recover

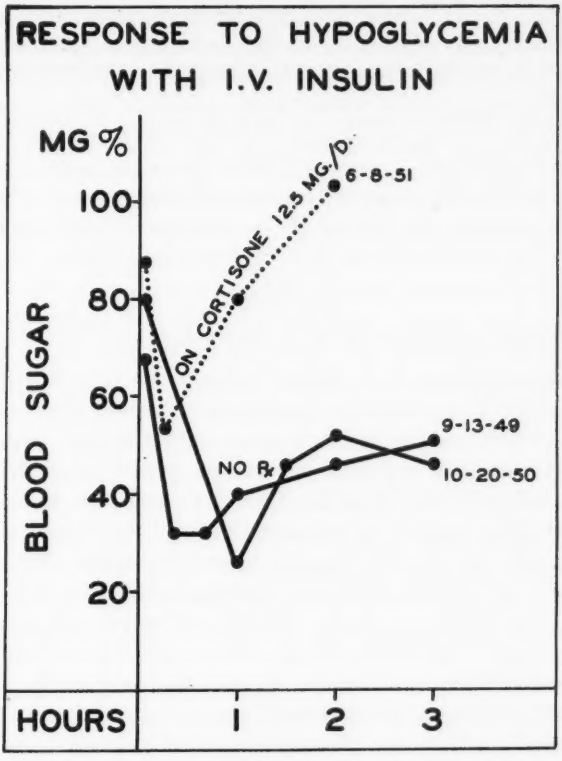

FIG. 7. Patient W. J. Before treatment with cortisone, intravenous insulin (0.1 unit per Kg.) caused hypoglycemia which persisted for three hours. With cortisone therapy, blood sugar rose to normal levels within one hour.

from hypoglycemia induced by intravenous insulin. In the two tests made while the patient was receiving no hormonal therapy, the blood sugar fell to levels of 26 and 46 mg. per cent and showed no significant rise after two and three hours. The test made while the patient was receiving cortisone is of the normal type, with a drop to 54 mg. per cent and a sharp recovery to 80 mg. per cent in one hour, and to 104 mg. per cent in two hours. These findings would be compatible with a deficiency of the glycogenetic hormones of the adrenal and replacement of their function by cortisone.

Before cortisone therapy was begun, 60 eosinophil counts were made at various times of the day, both when the patient was in comparatively good health and when he was severely ill with infections or cardiac failure. These ranged from 22 to 1,650 per mm. The majority of the counts were between 200 and 400 and the average for all counts was 434.

There was no definite decrease of the eosinophils during illnesses, infections and cardiac failure. There were not very definite responses during the injection of ACTH (April 1949 and September 1949) or during epinephrine tests (September 1950 and June 1951); the ACTH test, however, is difficult to interpret because perhaps the dose was too small. After continuous treatment with cortisone was begun in December 1950, the eosinophil counts ranged from 0 to 176 with an average of 77. Since eosinophil counts are affected by the glycogenetic group of adrenal hormones and cortisone, the findings would be compatible with an inability of the patient's adrenals to secrete adequate amounts of these hormones.

Before treatment, numerous determinations of the urinary "neutral-reducing lipids" (11-oxysteroids) revealed values from 0.08 to 1.21 mg. per day with an average of 0.34 mg. per day, which is a little lower than in most of our other cases of congenital adrenal hyperplasia. During the days immediately following the hypoglycemic episode of August 19, 1949, the output was 0.09 to 0.15 mg. per day. Values nearly this low, however, were found at other times when there were no hypoglycemic symptoms.

DISCUSSION

Relation of adrenal cortical hormones to hypertension

In the 3 cases of congenital adrenal hyperplasia described, suppression of adrenal activity by small doses of cortisone was associated with lowering of the blood pressure. This supports the hypothesis that the hypertension was caused by hormones of the adrenal cortex. In the case of J. S. there was a clear-cut relationship between the degree of adrenal androgenic activity, as measured by the 17-ketosteroid excretion, and the height of blood pressure. The blood pressure fell sharply when the urinary ketosteroids decreased, and it rose slightly during the fourth period when a decrease in the dose of cortisone to 50 mg. every third day (average, 16.6 mg. per day) permitted a return of adrenal activity with a rise of ketosteroid excretion. The blood pressure fell again when full adrenal suppression was attained by increasing the dose of cortisone to 75 mg. every third day.

In the case of M. C. S. the blood pressure did not fall sharply when the excretion of urinary ketosteroids was only partly suppressed by the administration of oral cortisone, but it was markedly reduced when full suppression was brought about by intramuscular therapy. The subsequent rise of blood pressure to hypertensive levels, which occurred during the third period of treatment when the patient was receiving 50 mg. of oral cortisone daily and 17-ketosteroids were being maintained at a low level, is more difficult to explain. It is possible that an excessive amount of cortisone was being given at this time. Against this is the fact that later the hypertension disappeared when the patient was receiving 25 mg. of intramuscular cortisone daily, because we have shown (6) that this dose given intramuscularly has as much or even greater action in suppressing 17-ketosteroid excretion than 50 mg. of oral cortisone. The rapid gain of weight which occurred simultaneously with the return of hypertension, and the

subsequent persistence of hypertension until a low sodium diet was instituted, suggest that salt retention played a role. Since salt restriction had failed to reduce the blood pressure before cortisone therapy was instituted, it appears that it was also necessary to suppress the overactivity of the patient's adrenal before hypertension decreased.

The case of W. J. is more complicated than the other two. During the first period of treatment at the age of 2½ years, the administration of 50 mg. of intramuscular cortisone daily resulted in increased hypertension, but it is not certain whether the action was a direct one or was due to cerebral stimulation. Subsequently the effects of 12.5 mg. of intramuscular cortisone daily were most spectacular. Unfortunately, this therapy was begun only when long-continued cardiac decompensation had resulted in what appeared to be a terminal fall of blood pressure. At this time the fact that the ketosteroid excretion had fallen to a low level suggested that adrenal activity may have been impaired as a result of prolonged illness, cardiac failure and poor nutritional state. There was also indirect evidence that in this patient there may have been a deficient secretion of glycogenetic hormones of the adrenal. The first action of cortisone seemed to be improvement of cardiac function, causing restoration of a normal systolic blood pressure. It is possible that cortisone served to replace glycogenetic hormones which were deficient and thereby improved metabolism and the nutritional state of the heart muscle. Following the improvement of cardiac function the systolic blood pressure did not rise to hypertensive levels and the diastolic pressure fell to normal levels. We attribute this result to the suppression by cortisone of the hypertensive factors of the adrenal.

The nature of the hormones responsible for hypertension which occurs in some cases of congenital adrenal hyperplasia is not known. The fact that hypertension can be caused by the administration of desoxycorticosterone suggests that it is related to salt retention or to changes in the electrolyte content of the cells. Cortisone can also cause hypertension, but the fact that large doses are required suggests that its action also is due to an effect on the electrolytes, since Thorn and his colleagues (7) have shown that 100 mg. of cortisone have an effect equivalent to that of 2 mg. of desoxycorticosterone. In none of our cases was any abnormality of serum electrolytes demonstrated, and in the one patient in which it was studied (W. J.) the blood volume was normal in relation to the size of the child. At the present time it is impossible to state whether or not the "pressor" factor of the adrenal cortex is identical with the "electrolyte-regulating" factor.

The histologic appearance of the adrenal cortex in the case of W. J. (Fig. 3) was of interest. Although the cortex was composed largely of cells resembling the zona reticularis, there was also a marked hyperplasia of the

zona glomerulosa. This is in striking contrast to the adrenals of patients with congenital adrenal hyperplasia who have a defect of electrolyte regulation, causing symptoms similar to those of Addison's disease. In these patients there is usually an absence of the zona glomerulosa (8, 9). Shepard and Clausen (4) have reported a patient with macrogenitosomia precox and hypertension whose hyperplastic adrenals showed marked enlargement of the zona glomerulosa, similar to that found in our patient. This finding is of interest in relation to the evidence offered by Deane and Greep (10) and by Jones (11) that the zona glomerulosa is concerned with the secretion of electrolyte-regulating hormones. Likewise, the theory that the carbohydrate-regulating hormones of the adrenal are derived from the zona fasciculata is compatible with the finding that this zone could not be well identified in the adrenal of our patient (W. J.), who apparently had a deficiency of these hormones.

Variations in the pathophysiology of congenital adrenal hyperplasia

In most cases of congenital adrenal hyperplasia the symptoms can be explained by the excessive secretion of androgen and there are no clinical evidences of an abnormality of electrolyte or carbohydrate regulation. In some of these cases, however, it appears that the administration of ACTH causes sodium loss rather than retention (12). The second group of cases consists of patients who, in addition to manifestations of excessive androgen secretion, have a disorder of the electrolyte regulation similar to that of Addison's disease. Unless properly treated these infants usually die at an early age. The therapeutic use of cortisone in addition to a high sodium intake, with or without desoxycorticosterone, will be discussed in another paper (13). A third variation in the symptomatic pattern of congenital adrenal hyperplasia is seen in the patients with hypertension. It is possible that a fourth group of patients might show a deficiency of carbohydrate-regulating activity of the adrenal. The evidence for such a deficiency is strongly suggestive but perhaps not conclusive in the case of W. J. This patient had only one attack of spontaneous hypoglycemia, and a comparatively long period of fasting (thirty-nine to forty-three hours) was required to reduce his blood sugar below the level of 50 mg. per cent. The oral glucose tolerance curves were comparatively flat. The induction of hyperglycemia by the intravenous injection of glucose was followed by a prolonged hypoglycemic phase. When hypoglycemia was produced by the intravenous injection of insulin, the blood sugar did not rise to normal after two to three hours. The administration of cortisone resulted in a return of the glucose tolerance and insulin curves to normal. The patient's eosinophil count showed a tendency to be high and did not fall with infections. The excretion of 11-oxysteroids was somewhat lower than that

which we have observed in our other cases of congenital adrenal hyperplasia. We know of only one other case in which it has been demonstrated that there was probably a deficiency of adrenal glycogenetic hormones. This patient, studied by White and Sutton (14), was a female pseudohermaphrodite of $4\frac{1}{2}$ years who had a urinary excretion of 4 to 9 mg. of 17-ketosteroids per day. There were frequent episodes of spontaneous hypoglycemia but no evidence of an electrolyte disorder. The outputs of 11-oxysteroids measured in our laboratory were 0.11 and 0.14 mg. per day, which are unusually low values.

SUMMARY

Three cases have been described in which congenital adrenal hyperplasia was accompanied by marked hypertension. In all cases the hypertension has disappeared during the administration of cortisone, and the blood pressure has been maintained at normal levels for periods of from nine to nineteen months at the present time.** For this purpose, it is necessary to give the small doses of cortisone required to suppress completely the activity of the abnormal adrenals. Larger doses of cortisone may elevate the blood pressure. The regulation of dosage of intramuscular and oral cortisone is discussed elsewhere (6). One of the patients with hypertension had hypertrophy of the zona glomerulosa which has not been noted in cases of congenital adrenal hyperplasia without hypertension. The relation of cortical adrenal hormones to hypertension is discussed.

One of the patients studied had evidences of deficiency of the glycogenetic hormones of the adrenal. Variations of the clinical symptomatology of congenital adrenal hyperplasia are discussed.

** *Addendum*

In July, 1952, as this paper goes to press, the blood pressures remain normal after fourteen to twenty-four months.

Acknowledgments

We wish to acknowledge the faithful assistance of Mrs. Mary Ellen Crafton, Mr. Henry M. Schulte, Jr. and Miss Betty Youngblood in performing steroid determinations.

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A CLINICAL LABORATORY PROCEDURE FOR THE STUDY OF THE GENERAL ADAPTATION SYNDROME*

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AT THE present time there is no simple clinical procedure which renders it possible to evaluate the intensity of the General Adaptation Syndrome of Selye (1) or to indicate when the reaction has been concluded. The present report presents a turbidity test applicable to blood serum and, with a slight modification, to urine, which renders such an evaluation possible. The proportions given have been found to be the most effective in producing the turbidity.

EXAMINATION OF SERUM

Materials

1. Nonhemolyzed serum is obtained by venipuncture at least four hours after a meal, and is diluted, 1 part of serum in 10 parts of distilled water; 0.5 ml. of serum is sufficient. It should be examined within twelve hours after its withdrawal.

2. Sorethytan-20-mono-oleate (Tween 80, Atlas Powder Co.)—20 ml. dissolved in 80 ml. of distilled water.

3. Ethyl alcohol (95 per cent)—100 ml. diluted with 20 ml. of distilled water.

Technic

Three test tubes are set up and the reagents added in the following order: distilled water—2 ml. in tube 1, 1 ml. in tube 2, and none in tube 3; the diluted alcohol—2 ml. in tube 1, 3 ml. in tube 2, and 4 ml. in tube 3; to each of the 3 tubes, 1 ml. of the diluted sorethytan-20-mono-oleate and 1 ml. of the diluted serum. The tubes are inverted to assure mixing and permitted to stand for thirty minutes at room temperature. The density of the turbidity is measured in a colorimeter (Klett-Summerson), using a 66 red filter.

Calculation

The reading of the galvanometer is recorded for each tube. The value obtained for the first tube is divided by 2, that for the second by 3, and that for the third by 4. These results are added and the grand total is divided by 3. The final average obtained represents the average degree of turbidity per ml. of alcohol and is referred to as the Turbidity Index (T.I.). Based upon 100 normal individuals, the average T.I. is 34, with a range of from 29 to 39. Inasmuch as the scales on various galvanometers vary, each worker should establish his own range of the normal.

In general, during the phase of alarm and the first period of adaptation, the patients were on a light diet.

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In normal individuals not subjected to stress the T.I. is constant for a period of at least seven days. The interpretation of the significance of T.I. values on a given patient is dependent upon whether there is a series of determinations or whether only a single determination has been made. We shall not present mass data, since a small series of cases illustrates the typical reactions with clarity. There are several other methods by which the body's response to injury can be measured, among them the temperature curve, blood count and red cell sedimentation rate. We have no extensive comparisons between these and the present method.

Cases

Case 1 (Fig. 1). Reaction to a single stress surgically inflicted. A cholecystectomy was performed for biliary calculi in a female aged 40. There was a smooth postoperative recovery. The point of surgical insult is indicated by S on the chart. Twenty-four

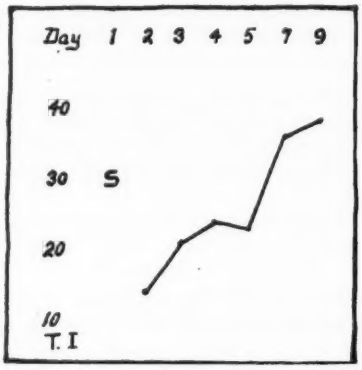

FIG. 1. Reaction to a single stress surgically inflicted (Case 1).

hours postoperatively the T.I. was 15. Forty-eight hours postoperatively the T.I. value commenced to rise, reaching 39 on the ninth day. The low T.I. values are considered evidences of the alarm; the rise in T.I. values, the adaptation phase. The highest T.I. value coincides with the completion of healing.

Case 2 (Fig. 2). Reaction to a single stress not surgically inflicted. A hypertensive arteriosclerotic male aged 60 had a coronary thrombosis at the point indicated by S on the chart. Twenty-four hours after the stress the T.I. was 34. Forty-eight hours after the stress it was 13 and remained at this level until the sixth day. The value dropped to 10 on the seventh day but two days later started to rise, finally reaching 32 on the forty-sixth day. In this instance the alarm reaction was delayed and the adaptation process was long drawn out.

Case 3 (Fig. 3). A single nonsurgical stress followed by exhaustion instead of adaptation. A nonarteriosclerotic male aged 45 had a moderately severe coronary thrombosis at the time indicated by S on the chart. The alarm reaction was immediate, the T.I.

being 17 within twenty-four hours after the stress. The eighth day after the stress the T.I. was 6. The patient died suddenly on the tenth day and a T.I. taken four hours ante mortem, when death did not appear to be imminent, was 3.

Case 4 (Fig. 4). A stress occurring some time prior to hospitalization, followed by two

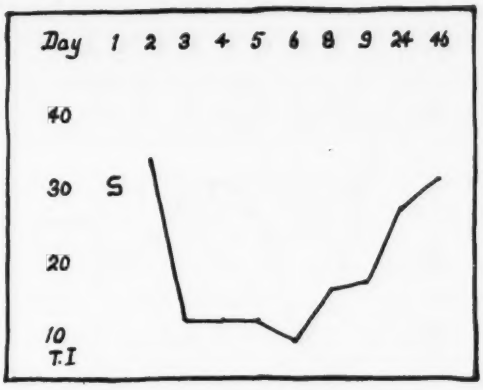

FIG. 2. Reaction to a single stress not surgically inflicted (Case 2).

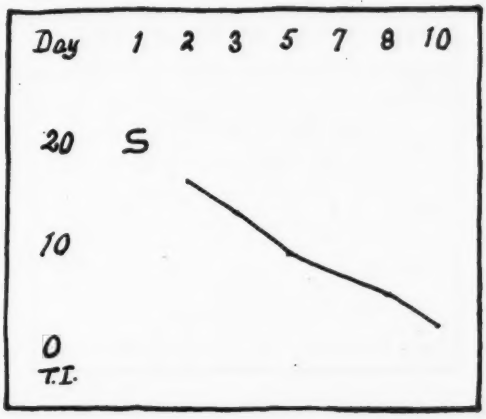

FIG. 3. A single nonsurgical stress followed by exhaustion instead of adaptation (Case 3).

stresses surgically inflicted, and adaptation to all three. A male aged 55 had an adenocarcinoma of the sigmoid not giving rise to complete obstruction. On admission the T.I. was 48. High T.I. values indicate that the organism is making violent efforts to attain adaptation. One can refer the initial high value in this instance to the tumor. A colostomy (the first S in Fig. 4) was performed on the second day of hospitalization. Twenty-four hours later the T.I. was 29 and the following day, 20. Five days postoperatively the T.I. had risen to 36, indicating that adaptation to the surgery had been accomplished.

Twelve days after the colostomy the T.I. was 42, indicating that the adaptation to the tumor was still in progress. The tumor was resected (the second S in Fig. 4) on the fifteenth day. This was followed by a fall in the T.I. for eleven days; then the Index commenced to rise toward normal values, at which point it became stationary. The stress

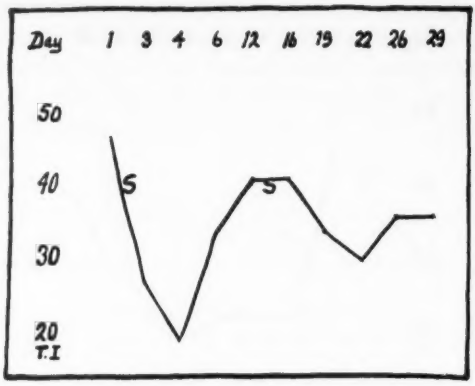

FIG. 4. Adaptation to multiple stresses (Case 4).

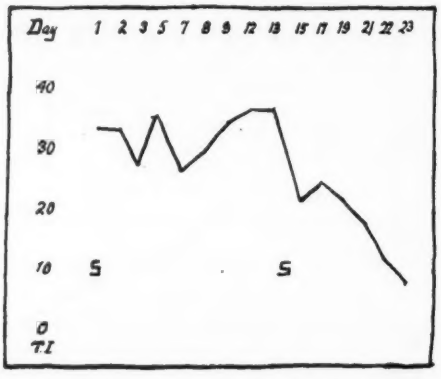

FIG. 5. Exhaustion following multiple stresses (Case 5).

of the second surgical procedure had been overcome and the surgery, in turn, had removed the stress occasioned by the tumor.

Case 5 (Fig. 5). A case similar to Case 4, but with exhaustion instead of adaptation. A male aged 60 had acute intestinal obstruction due to a carcinoma of the transverse colon. On admission the T.I. was 34. A cecostomy was performed a few hours after admission (the first S in Fig. 5). Twenty-four hours later the T.I. was still 34; forty-eight hours later, 27; four days later, 36; and twelve and thirteen days later, 38. On the fourteenth day of hospitalization the second surgical insult, resection of the tumor (the second S in Fig. 5), was inflicted. On the fifteenth day the T.I. was 22. The value of the Index continued to fall and the patient died eleven days after the second operation. The terminal T. I. was 8.

Case 6. The effect of multiple successive stresses with an accumulative adaptative reaction is illustrated by this case. In a male aged 52, urinary retention of several months' duration had become acute. The patient had had syphilis but a serologic test gave negative results. He also had a quiescent duodenal ulcer. His admission T.I. was 58. He was cystoscoped and the following day the T.I. was 49. On the eighth day of hospitalization a transurethral prostatectomy was performed; the T.I. on that day was 68. For the next three days the T.I. was 61. It then rose to 72 and concurrently the patient complained of a hemorrhoid. The Index value then rose until on the seventeenth postoperative day it was 106. The hemorrhoid was then incised and evacuated. Three days later the T.I. was 52 and on the twenty-second day of hospitalization, 35.

The interpretation of single Index determinations is slightly less certain than in the cases just cited. In general, values below 25 indicate either an alarm or lack of adaptation. The lower the value the more severe the stress or the less effective is adaptation. Values above 40 indicate that although adaptation is taking place, the organism is having difficulty. The following are the results in a series of single T.I. determinations: in 8 cases of cirrhosis of the liver, 4 of which were associated with jaundice, 89, 106, 48, 65, 74, 80, 72; in 3 cases of jaundice due to obstruction by calculi, 28, 25, 23; in 3 cases of leukemia, 53, 68, 68; in 1 case of myasthenia gravis, 64; in 1 case of carcinoma of the ovary, 59; and in 1 case of malignant tumor of the brain, type not determined, 64.

Different types of stress and different degrees of adaptation acting on the same organ give differing single T.I. values, *e.g.*, in acute pancreatitis, 14; in chronic pancreatitis, 24; in carcinoma of the pancreas, 68; in nonsclerotic hypertension, 31; in moderately advanced arteriosclerosis, 20; in fully compensated quiescent rheumatic heart disease, 28; in acute cardiac decompensation, 10; in marked myocardial insufficiency, 55; in rheumatoid arthritis, 33; and in gout, 66.

EXAMINATION OF THE URINE

For use on urine the technic has been modified as follows.

Materials

1. Ethyl alcohol (95 per cent)—100 ml. diluted with 20 ml. of distilled water.
2. Sorethytan-20-mono-oleate (Tween 80, Atlas Powder Co.)—20 ml. dissolved in 80 ml. of distilled water.
3. Sodium hydroxide—10 per cent aqueous solution containing a small amount of sodium carbonate.

Technic

Four ml. of alcohol and 1 ml. of the Tween-80 solution are placed in a test tube and 0.1 ml. of the sodium hydroxide is added. A turbidity develops in from ten to twenty minutes. The tube is permitted to stand at room temperature until the turbidity clears (eight to twelve hours). The tube is now ready for use at any convenient time. The turbidity having cleared, 1 ml. of the selected urine is added and the tube inverted. Again a

turbidity develops. The degree of turbidity is measured in a colorimeter (Klett-Summerson), using a 66 red filter. The reading of the galvanometer is recorded as the unit of turbidity. To distinguish the value from that of the blood it is termed Urinary Turbidity Index (U.T.I.).

The colorimeter should be standardized against a tube of the reagent which has cleared but to which no urine has been added. Neither the color of the urine nor its concentration has any significant effect on the U.T.I. and strongly bloody or bile-stained specimens can be used. Cloudy specimens not containing gross clumps can also be used. The presence of albumin or sugar is also immaterial. The Index values obtained with urine are at a higher level than those recorded for serum and although trends of behavior can be compared, the numerical values cannot.

Normal individuals not subjected to stress show Index values ranging from 50 to 150. Specimens voided at different periods during the day usually have different Index values. The peak values commonly occur in the late afternoon or evening. In the clinical work reported here, specimens voided in the early morning and evening were examined and the average Index for the two was taken as the Index for the day. Twenty-four-hour specimens serve the same purpose.

The U.T.I. values for a normal individual not subjected to stress and covering a period of four days are fairly constant. In an individual who on the second day of observation was subjected to four hours of intense mental effort and tension, the Index rose sharply and did not return to the normal range for twenty-four hours after reaching a peak. Whereas stress or alarm causes the Index of the blood to fall, it causes the Index of the urine to rise.

In 2 cases of coronary thrombosis the stress (coronary thrombosis) occurred five days before the first U.T.I. was determined, the initial Index was above normal in both, and on the ninth day it dropped to within the normal range. The values stayed within that range for three days and then rose slightly.

In 2 cases of cardiac decompensation with congestive failure the pattern of the curves was followed over a considerable period, although only the terminal portions are shown in Figure 6. One of the patients had rheumatic heart disease; the other had hypertensive heart disease. For a period of ten days the Index values ranged between 275 and 300. In both cases a peak value (400 in one, 390 in the other) was reached several days before improvement in compensation was clinically definite. The peak having been reached, the values fell progressively; and coincidental with the fall there was continuous clinical improvement.

In a series of 60 single determinations of the Index on 60 different individuals (evening specimens being examined), 10 of whom had rheumatic heart disease in a completely quiescent state and 50 of whom had arteriosclerotic heart disease, we were not able to establish any relationship

between the Index value and the type or location of the lesions. The rheumatic heart cases had values under 150; the arteriosclerotic group had values above 150 and ranging to 390.

A patient with rheumatic fever during a period of twenty days received 4,100 mg. of cortisone. The patient was symptom-free and the medication was discontinued on the day the second Index determination was made. The Index values were fairly constant for seven successive days. On the

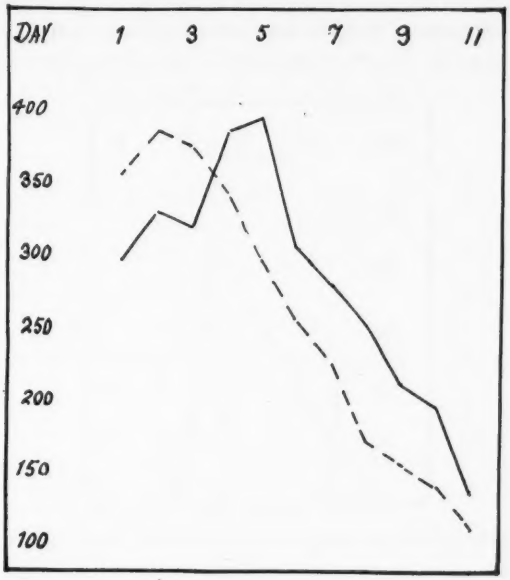

FIG. 6. Index values in cardiac decompensation with congestive failure. The solid line represents rheumatic heart disease and the broken line, hypertensive heart disease.

eighth day there was a decrease in the value, which decrease was further extended on the ninth day. Coincidentally, there was a recurrence of symptoms. The recurrence was treated with salicylates. The values rose and on the twelfth day reached a maximum. Coincidentally the symptoms subsided. The Index subsequently fell to 100. In this instance the curve was contrary to the rule. We have no explanation for this reversal.

Figure 7 illustrates the reaction to physiologic stress. The observations were made on a woman before and after labor. The day before the onset of labor the Index was 98, twenty-four hours after delivery it was 150, four days post partum it was 280, and seven days post partum it was 80.

The reaction to stress surgically inflicted is shown in a patient subjected

to a prostatectomy for urinary retention. He was cystoscoped two days before the first Index was determined; the value was 260. The Index fell, until on the fifth examination it was 130. The prostatectomy was performed at this time. Following the surgery the Index rose in steplike fashion to a peak of 480, attained nine days after the operation. In the ensuing three days it fell to 160.

The reaction in malignant tumors is shown by 2 cases. In both there was a double stress *i. e.*, the tumor and the surgical procedure. One of the cases was a carcinoma of the bladder; removal was not possible. The patient made a surgical recovery from the exploratory procedure, and the

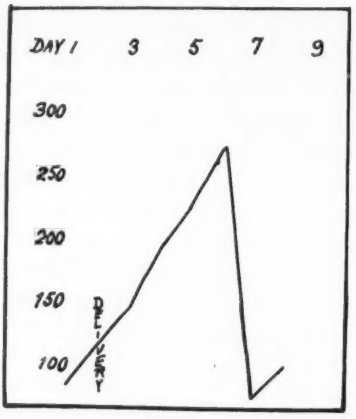

FIG. 7. Reaction to physiologic stress in a woman, before and after labor.

first Index was determined on the sixth postoperative day. The other case was an ulcerating carcinoma of the breast, for which a simple mastectomy was performed. The patient made a surgical recovery, and the first Index was determined on the fifth postoperative day. The curves were similar in character to those seen in coronary disease. The stress having been inflicted, it was followed by an increase in the Index value. As healing occurred the Index value fell. The opposite situation exists in the blood; there, the stress having been inflicted, the Index value drops and as adaptation occurs, the Index value rises.

The reaction in 2 other cases of neoplastic disease is also presented. One was a case of bronchiogenic carcinoma which was explored but not removed. The preoperative Index was 135; on the third postoperative day it had risen to 405 and eight days later it was 155. This pattern is characteristic of stress followed by full and complete adaptation. The second case was that of a small adenocarcinoma of the rectum, for which an abdominal

perineal resection was performed. The subsequent clinical progress was without untoward incident, until a pulmonary embolism caused death on the fourteenth postoperative day. The preoperative Index varied from 110 to 205. On the third postoperative day it was 450 and on the fourth it fell to 340. It then rose to 395 and fell to 315, but two days later it was 465. The value then declined, had a secondary rise, and fell abruptly to 180. If the pattern had been normal, the first fall in value would have continued. The case is presented as illustrating difficulty in attaining adaptation, a finding which can be taken clinically as a warning of impending complication. In this instance the warning came as early as the fifth postoperative day, nine days before the complication was clinically recognizable.

SUMMARY

A laboratory procedure applicable to blood and urine, which renders it possible to evaluate the General Adaptation Syndrome of Selye, is reported. A series of cases is presented illustrating the clinical application of the procedure.

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MODIFICATION OF RESPONSE TO PROLONGED ACTH THERAPY*

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EVANS (1), in 1924, reported that hypertrophy of the adrenals resulted from injections of anterior pituitary extracts. This observation stimulated much work in this direction (2–5), culminating in the successful isolation of a purified corticotropin (6, 7). Browne (8), in 1943, employing minute quantities of ACTH, recorded certain metabolic and hormonal changes in 2 normal human subjects. Thorn *et al.* (9), in 1947, studied a case of panhypopituitarism in which a "prolonged" course of ACTH had been administered, *i.e.*, 40 mg. per day for six days. In 1948, Mason *et al.* (10) and Forsham *et al.* (11) presented their studies on the metabolic and hormonal changes observed in normal subjects and in patients with various pathologic conditions (Addison's disease, chromophobe adenoma of the pituitary, and anorexia nervosa) following extensive use of ACTH. Many important studies soon followed (12–17).

In 1950, Thorn *et al.* (12) said, "It is possible to stimulate a relatively inactive (adrenal) gland to high levels of activity by prolonged therapy" and "refractoriness to the ACTH preparations of a particular species may develop." A search of the literature failed to reveal any prolonged study for the purpose of ascertaining whether or not a patient becomes refractory, or whether high levels of adrenal cortical stimulation can be maintained by prolonged therapy.

The purpose of this study has been to answer two questions: Can a stimulated adrenal cortex be maintained at high levels of activity with prolonged ACTH therapy? Does refractoriness to ACTH develop? Since the secretion of ACTH in panhypopituitarism is negligible or entirely absent, we selected for this study 2 patients with panhypopituitarism resulting from a chromophobe adenoma. This eliminated, as much as possible, the presence of endogenous ACTH production and, in turn, endogenous cortical output.

CLINICAL ABSTRACTS

M.M., a white male, aged 23, was first seen by our group in 1948 in consultation with the medical staff of the U.S. Army Hospital (Oliver General), Augusta, Georgia. The patient had served in the U.S. Navy from 1942 until 1946, seemingly performing normal

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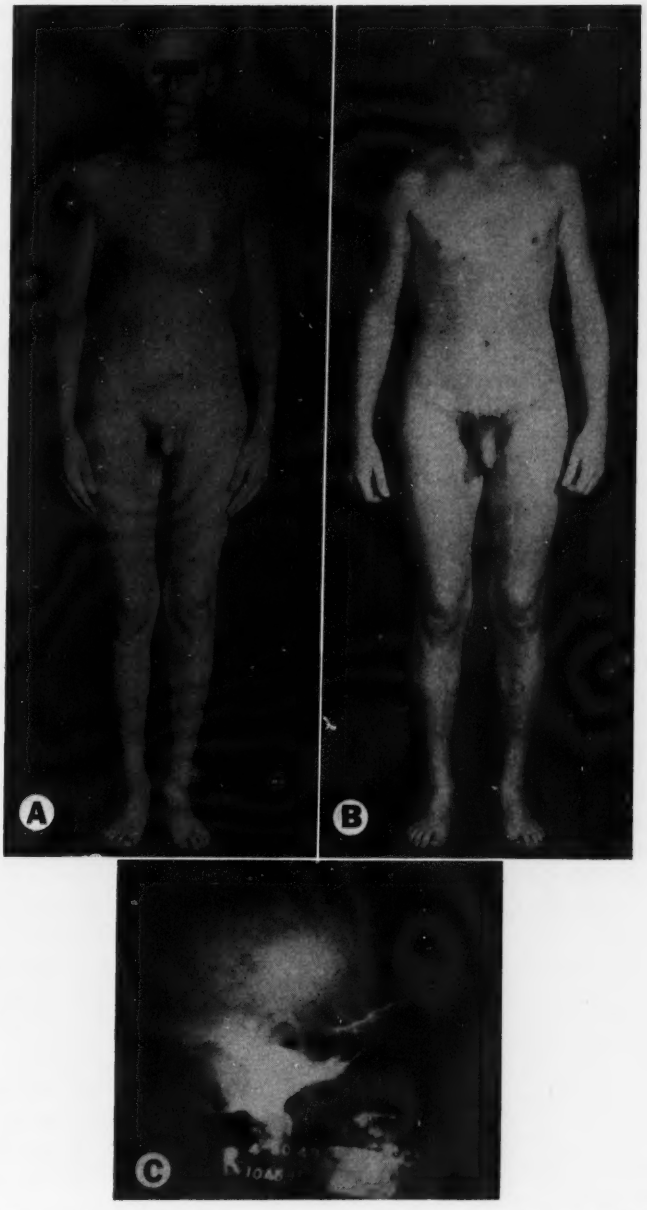

FIG. 1. Patient M.M. A. Note complete absence of pubic hair. B. After androgen therapy—return of pubic hair. C. Lateral view of skull, showing the marked ballooning of the sella turcica.

duties with efficiency. After discharge from the Navy, he enlisted in the U.S. Air Force. Soon after enlisting he began to complain of weakness, anorexia, weight loss, headaches, loss of libido and potentia, loss of beard, body and axillary hair, episodes of perspiration and fainting, and marked pallor.

Physical examination revealed skin that was very pale and of very fine texture. Linear markings were very evident around the lips and forehead. The eyebrows were thin and there was no beard. Body and axillary hair were absent. The testes were small (Fig. 1 A).

Laboratory studies: A roentgenogram (Fig. 1 C) showed the sella turcica to be extremely enlarged (28 mm. in its anteroposterior diameter and 23 mm. in its vertical diameter). There was considerable thinning and erosion of the dorsum sellae and posterior clinoid, and the floor of the sella was depressed to a pronounced degree. A Robinson-Kepler-Power water test gave positive results. Examination of semen showed aspermia. An insulin tolerance test (one-half dose) gave the following results: fasting blood sugar 102 mg. per cent; 20 mins., 38 mg.; 30 mins., 38 mg.; 45 mins., 50 mg.; 60 mins., 52 mg.; 90 mins., 70 mg.; 120 mins., 76 mg. per cent. A glucose tolerance test showed: fasting blood sugar, 80 mg. per cent; 30 mins., 118 mg.; 1 hr., 90 mg.; 2 hrs., 70 mg.; 3 hrs., 82 mg.; and 4 hrs., 79 mg. per cent. The basal metabolic rate was −25 per cent. Urinary 17-ketosteroids were 3.5 mg. per twenty-four hours. The results of a complete blood count, urinalysis, serology, and vision and visual fields tests, were all within normal limits.

With the foregoing history and laboratory data, a diagnosis was made of panhypopituitarism secondary to a chromophobe adenoma.

Radiation to the tumor and supportive hormonal therapy restored this patient to a fairly normal life. Pubic and axillary hair returned (Fig. 1 B) but loss of potentia was still evident. Therapy was maintained by our group until four months prior to this study, at which time all therapy was withheld. The patient's condition slowly regressed, with a return of some of the original complaints, *viz.*, lessening of appetite, headaches, weakness, weight loss and pallor. Decrease in pubic and axillary hair was not observed.

The patient lived under a normal regimen during the period of study. Food and water intake was unrestricted. He was employed by the Medical College of Georgia as a technician after his retirement from the service and had a good understanding of the importance of accurate 24-hour collections of urine. Any loss due to spilling or lack of a container (which occurred several times) was brought to our attention.

H.H., a white female, aged 34, was referred to us for diagnosis and endocrine survey in March 1951. She had had an uneventful childhood, but about the time of puberty she began to have spells of weakness. The scanty growth of pubic and axillary hair disappeared. There were a few occasions when slight spotting occurred, but the patient had never experienced a true menstrual period. It was therefore estimated that the disturbance had existed for at least twenty years.

Physical examination revealed an apparently well nourished female who appeared normal in a weight-height relationship (Fig. 2 A). The skin was of a fine texture and was cool, dry and very smooth. Linear markings were evident around the mouth, eyes and forehead. The breasts were small and adolescent. The thyroid was not palpable. There was complete absence of axillary and pubic hair and the genitalia were of the adolescent type. The vaginal smear showed only castrate cells.

The chief complaints of the patient were slight headaches, weakness, amenorrhea, increased numbness and stiffness of the right arm, leg and tongue, and polydipsia and polyuria.

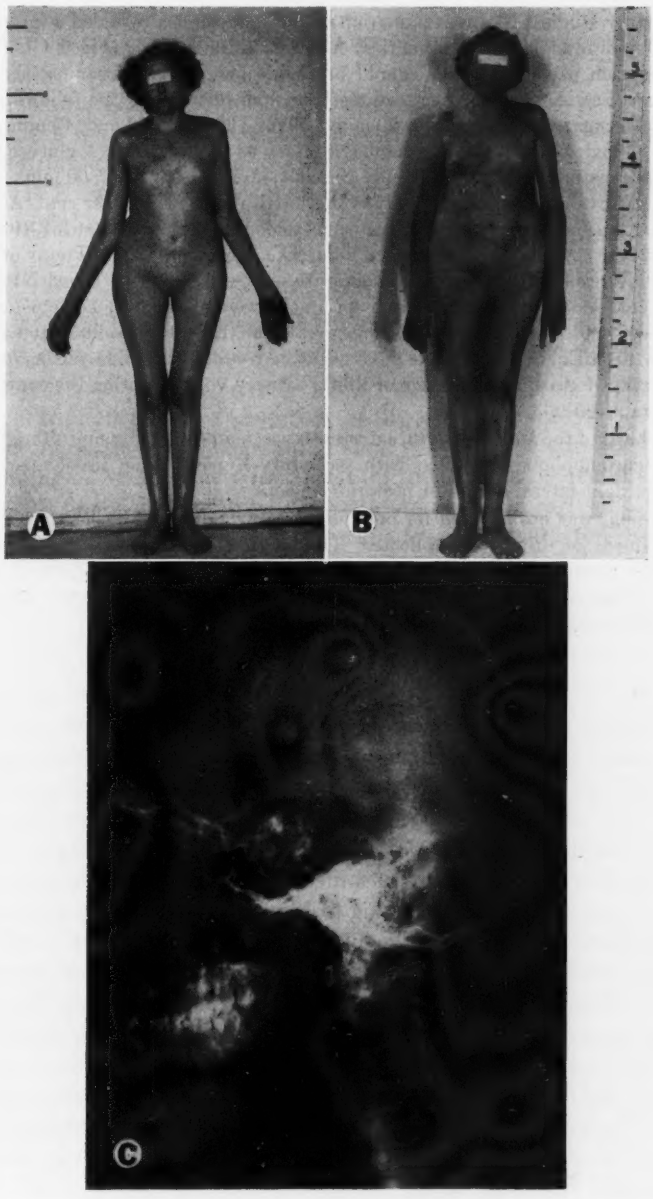

FIG. 2. Patient H.H. A. Note extreme sexual infantilism. B. General improvement in body contour and breast development after ACTH therapy. C. Lateral view of skull, showing large calcified pituitary tumor with marked erosion.

Laboratory studies: X-ray examination of the sella turcica revealed a large calcified tumor, 40 mm. in diameter, with marked enlargement of the sella (Fig. 2 C). There was extreme erosion of the posterior clinoid processes and partial erosion of the anterior clinoid processes. An insulin tolerance test (one-half dose) gave the following results: fasting blood sugar, 91 mg. per cent; 20 mins., 50 mg.; 30 mins., 52 mg.; 45 mins., 56 mg.; 60 mins., 75 mg.; 90 mins., 80 mg.; and 120 mins., 93 mg. per cent. A glucose tolerance test showed: fasting blood sugar, 95 mg. per cent; 30 mins., 139 mg.; 60 mins., 127 mg.; 2 hrs., 98 mg.; 3 hrs., 87 mg.; 4 hrs., 111 mg.; and 5 hrs., 91 mg. per cent. A complete blood count was normal except for 6 per cent eosinophils. An adrenalin test (four hours) showed 621 to 481 eosinophils per cu.mm. The Robinson-Kepler-Power water test gave positive results. The basal metabolic rate was −15 per cent. Blood NPN was 24 mg. per cent. Assays on 24-hour urine specimens were as follows: 17-ketosteroids, 6.1 mg.; corticoids, 0.14 mg.; creatine, 250 mg.; creatinine, 1,290 mg.; sodium, 100 mEq.; and potassium, 48 mEq. Serologic tests gave negative results. The urinalysis gave negative findings except for a specific gravity of 1.003. Urinary volume during the control period varied from 2,500 to 3,500 cc.

On the basis of the foregoing data, a diagnosis was made of panhypopituitarism secondary to a chromophobe adenoma, with secondary hypothalamic involvement of the supraoptic, right lateral and right posterior nuclei.

The patient was hospitalized for one hundred days, during which time accurate 24-hour specimens of urine were collected. The specimens were processed immediately. If it was not possible to proceed with the work on a specimen at once, it was refrigerated at 3–5° C. The patient was given a normal hospital diet, and no attempt was made to regulate electrolyte intake.

METHODS AND MATERIALS

Hematologic studies were made on oxalated venous blood, using a balanced oxalate solution. Blood counts were carried out by routine methods. Eosinophil counts were calculated by a modification of the method of Dunger (18) as described by Thorn *et al.* (9). 17-Ketosteroids were extracted according to the method of Pincus (19). Formaldehyde-liberating substances (corticoids) were determined by the method of Daughaday (20), with minor modifications according to Mason (21). Creatine and creatinine determinations were made by the method of Folin (22). Urinary sodium and potassium were determined with a Beckman DU flame photometer.

ACTH (Armour) was used in this study. The ACTH was dissolved in sufficient amounts of sterile water to allow the crystals to dissolve. The gluteal regions were used as the site of administration. Five-tenths of a cc. of a 2 per cent procaine hydrochloride solution was used to alleviate any pain from the injection. The procaine solution was administered first and then, after changing syringes, the solution containing the ACTH was injected into the same area.

Cortisone, 25 mg./cc. in aqueous suspension, when employed, was injected into the opposite gluteal area.

Concomitant thyroid therapy was given to Patient M.M. from the

fiftieth day to the end of the study, and to H.H. throughout the entire period of observation.

In Patient M.M., two equally divided doses of ACTH were given daily. The first dose was given at 9 a.m., the second at 5 p.m. M.M. received a total of 9,615 mg. of ACTH and 1,000 mg. of cortisone.

Patient H.H. received three equal daily doses of ACTH at 8 a.m., 4 p.m. and 12 midnight, respectively. Total ACTH administered to this patient was 5,900 mg.

RESULTS

Figure 3 (M.M.) and Figure 4 (H.H.) show graphically the results of the laboratory studies on both of these patients.

Well being. Both subjects responded almost immediately to ACTH therapy, in that there was an increase in the general feeling of well being. M.M. was much slower in responding than was H.H. It can be seen that the well being of M.M. slowly increased up to the time he began the combined ACTH-cortisone therapy. With the addition of cortisone to daily therapy, M.M.'s feeling of well being declined rapidly over a ten-day period (days 25 through 35); but with the discontinuance of cortisone, remaining on the same dosage of ACTH, this feeling of well being immediately improved. The only period of euphoria seen in M.M. during the entire study was from the fortieth to the fiftieth days of the study. During this euphoric stage, his appetite increased tremendously. His skin assumed a normal coloration. His mental outlook was changed decidedly for the better. Previous to treatment, his approach to work was rather lackadaisical, but during this ten-day period all work was approached with zeal. He exhibited a "joie de vie" never before seen by any of his co-workers. Libido increased to the highest point in the patient's memory. Insomnia troubled the subject but his feeling of well being so overshadowed this that he dismissed it as a point of little concern.

With H.H. there was an immediate response to ACTH. Within six days she was euphoric. With the onset of edema, well being was diminished. With the alleviation of this edema her feeling of well being again increased, without, however, the euphoria.

Urinary 17-ketosteroids. 17-Ketosteroid levels in both subjects increased on ACTH therapy. M.M. showed a threefold increase within ten days after the start of therapy. It is of interest to note that when M.M. was taking 40 mg./day of ACTH plus 100 mg. of cortisone, the 17-ketosteroid levels were the highest in the entire study. This will be discussed later. As the study progressed, response became progressively less, insofar as 17-ketosteroid production was concerned. The same dosage of ACTH in the later part of the study failed to elicit the same response as it did in the early part of the study.

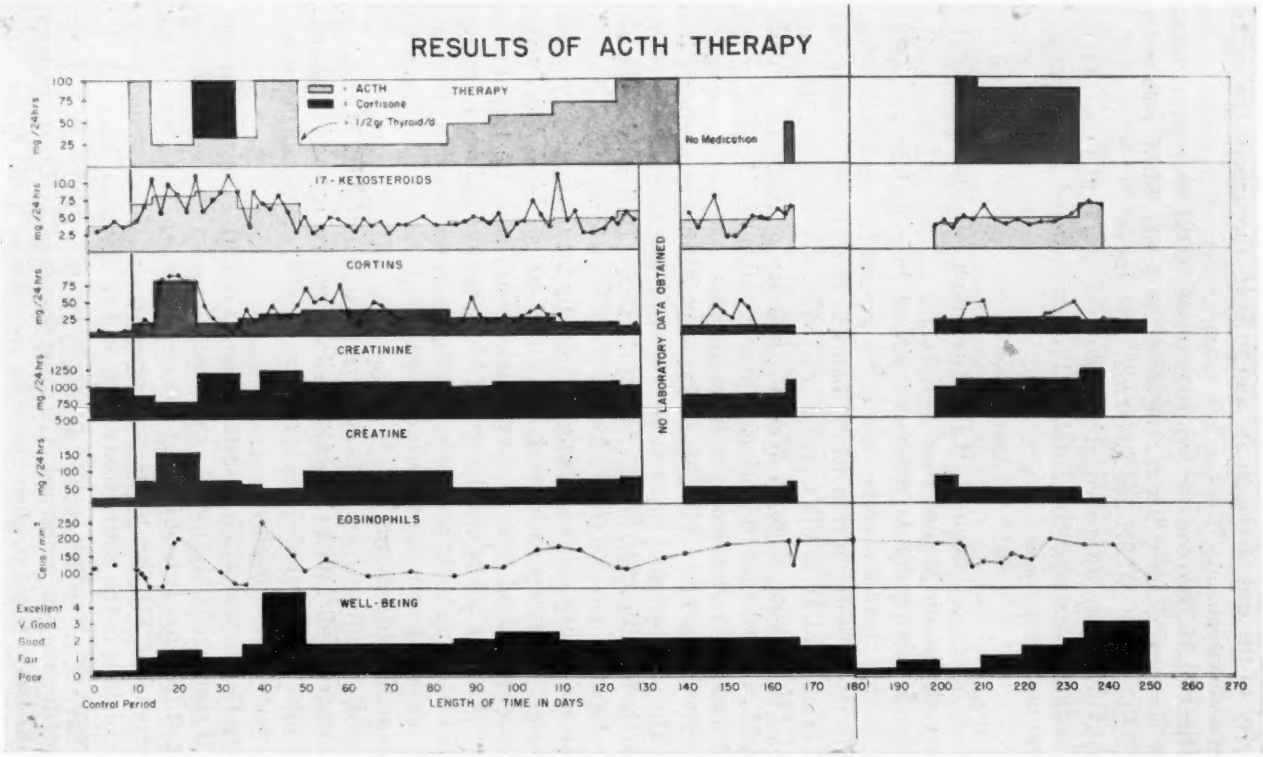

FIG. 3. Results of study on Patient M.M. As this subject was on a constantly changing dosage schedule, the solid background for the various determinations has been averaged for each particular dosage level. The connected lines in the 17-ketosteroid and cortin graphs represent 2-day averages of assays.

H.H. was much slower in responding to ACTH and it was not until the twenty-fifth to the thirtieth days that an increase was noted in 17-ketosteroid output. After attaining this increase, the 17-ketosteroid output appeared to decrease from the fiftieth day onwards. In spite of continuation of 50 mg. of ACTH per day from the fifty-fifth to the eighty-second days, the urinary 17-ketosteroids gradually decreased to pretreatment levels.

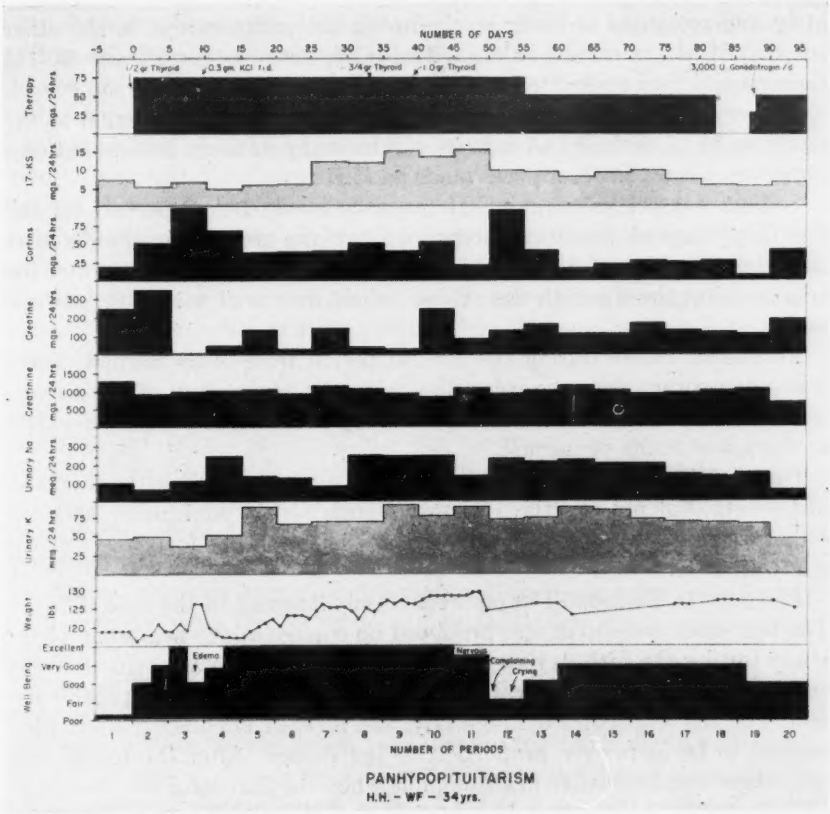

FIG. 4. Results of study on Patient H. H. This subject was on a constant dosage schedule; therefore the entire study was broken down into 5-day periods to show the changes in an orderly fashion.

Urinary corticoids. Corticoid levels in both subjects showed an average increase of about fivefold (H.H.) to tenfold (M.M.) within the first ten days of therapy. These values are in rough agreement with those of Ven-

ning *et al.* (15), although our values show that with continued administration of the hormone, there is a later trend to levels which are much lower than the peak. The levels, nevertheless, remain higher than during the control period.

Urinary creatine. Creatine levels rose initially from abnormally high levels, but in both subjects tended to fall to lower levels during therapy, indicating better muscle metabolism of this product.

Urinary creatinine. In one subject (H.H.), creatinine values fell immediately and remained at lower levels during the entire study. In the other subject, M.M., creatinine values fell initially, but rose on cortisone-ACTH therapy and then tended to remain at a slightly higher, or on the same level.

Urinary sodium and potassium. Unfortunately, the first portion of the study on M.M. included no data on sodium and potassium determinations. However, a complete study was made on H.H.

Sodium control values on H.H. were decidedly below normal for the first thirty days of the study, except for a five-day period immediately after the administration of 0.3 Gm. of KCl t.i.d. was started for control of the edema. After the thirtieth day, these values were well within the normal range.

Potassium values during the control period were below normal. There was a temporary decrease coinciding with the appearance of the edema, but oral KCl for the remainder of the study maintained potassium values at the lower limits of normal.

Urinary chlorides. Chloride values in both subjects paralleled sodium values closely, but not exactly. Because of such a close conformity with the sodium results, this test was followed for only the first forty days of the study on H.H.

Eosinophils. Eosinophil levels were closely followed in the case of M.M. The four-hour eosinophil test produced no change at the beginning of the study but by the fifth day there was a drop from 80 to 50 eosinophils per cu. mm., showing that cortical stimulation was beginning to approach normal. For the remainder of the study, the level of circulating eosinophils seemed to be in inverse proportion to the dosage. After the ninety-fifth day, there was no change in eosinophils when the four-hour test was made, and increasing the dosage did not elicit a good response by way of reducing the number of circulating eosinophils.

In patient H.H., eosinophil values were high (*e.g.*, 621) during the control period. After ten days of ACTH therapy, these values had not been markedly reduced. A stool examination revealed the presence of many ova and parasites of the hookworm. Because of the parasitic complication, eosinophil responses in this case were not valid and were thus excluded from the study.

General findings. In subject H.H. the feeling of numbness and stiffness of the right arm, leg and tongue quickly disappeared. By the fifth day it was easy for her to use all affected parts, whereas previously there had been restriction of flexion of the right hand and dorsoflexion of the toes of the right foot. This condition, about six weeks after the end of the study, returned to the status prior to treatment. Following further steroid therapy however, there was marked improvement.

There was no elevation of blood sugar or of blood pressure in either subject during the entire study. M.M. exhibited no edema, and neither patient showed any evidence of Cushing's disease.

Physical examination of H.H. on the thirty-third day of therapy revealed the presence of a fuzzy new growth of pubic hair. The patient also complained of soreness of the breasts and felt that their size had increased, although the change did not seem definite to the examiner. There was no change in vaginal cytologic pattern during the entire study; it remained atrophic. Very slight pubic hair growth became evident as the study progressed. The hair, however, was very fine and not readily visible. Physical examination of this patient on the last day of therapy revealed a woman with much better proportions (Fig. 2 B) and with a definite increase in breast development.[1]

DISCUSSION

During the period when M.M. was receiving cortisone and ACTH, urinary cortin (corticoid) values fell but the 17-ketosteroid values rose to even higher levels than on the previous dosage schedule of 30 mg. of ACTH per day. Sprague *et al.* (17) suggest that some functions of the cortex can be depressed with prolonged cortisone production. They also feel that the 17-ketosteroids excreted during administration of cortisone are derived largely, if not entirely, from the cortisone administered. Our study seems to confirm the validity of this statement.

Examination of the findings on M.M. show that about the 110th day, increase in dosage of ACTH did not elicit a corresponding increase in the 17-ketosteroid and cortin values, and that by the 130th day, another substantial increase in dosage from 75 to 100 mg. a day failed to change the upward course of the eosinophil count. After a 32-day rest period, a second

[1] On the last day of the study on subject H.H., pellets were implanted. This included two pellets (75 mg. each) of cortisone, one pellet of Cortate (75 mg.), and one pellet of progesterone (50 mg.). Thyroid was continued in a dose of 1 grain daily, along with one tablet daily of a combination of methyltestosterone (10 mg.) and estradiol (1.0 mg.). There was some immediate edema, which was controlled with potassium chloride. Within two months there was a definite growth of pubic hair, indicating that exogenous ACTH therapy did not stimulate production of steroids sufficiently for the development of secondary sexual hair growth. Axillary hair was still not evident.

intensive course of ACTH was given. Response was slight in comparison to the values found in the early portion of the study.

In the case of H.H. (Fig. 4) corticoid production was immediate. This is an excellent illustration of the rebound phenomena of Thorn *et al.* (12), namely, stimulation of the cortex, overproduction of corticoids, and then a fall to levels between the previous low and high points. The reverse was true when the dosage schedule was lowered, as is well illustrated in this patient.

In the case of M.M. there was an immediate response both in 17-ketosteroid and corticoid production, but with H.H. there was no immediate response in 17-ketosteroid production. We are at a loss to explain this lack of response unless it can be postulated that there is a selectivity of action of ACTH on the cortex, as suggested by Sprague *et al.* (17). This problem will be the subject of further investigations.

In these cases of panhypopituitarism it was expected that the subjects would show high urinary sodium loss due to a secondary hypofunction of the adrenal cortex. In the light of the control sodium values on H.H., it can be assumed that the sodium intake was low during this period.

From the data obtained in this study, we do not feel that a stimulated adrenal cortex can be maintained at high levels of activity with prolonged ACTH therapy. Forsham *et al.* (23) believe that tissue inactivation of ACTH, rather than an antihormone factor is responsible for the resistance, although they found that switching from pork ACTH to sheep or beef ACTH renews adrenal activity. Administration of ACTH intravenously by slow drip (20 mg. during four to eight hours) was also found to renew adrenal activity.

SUMMARY

The hormonal and metabolic changes resulting from prolonged ACTH therapy in 2 cases of panhypopituitarism secondary to chromophobe adenoma are reported.

One patient received 9,615 mg. of ACTH plus 1,000 mg. of cortisone over a period of two hundred and seventy days. The other patient received 5,900 mg. of ACTH over a period of one hundred days.

It is the opinion of the authors that prolonged administration of ACTH induces modification of adrenocortical responsiveness. It is felt that a stimulated adrenal cortex cannot be maintained at high levels of activity with prolonged corticotropin therapy.

Acknowledgments

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THE EFFECT OF SURGICAL PROCEDURES ON URINARY GONADOTROPIN EXCRETION

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IN A recent publication (1), we reported excessive urinary gonadotropic activity in certain patients receiving cortisone or pituitary adrenocorticotropin (ACTH) therapy. This occurred in 9 of 22 subjects who were treated with these agents for a variety of diseases. The nature of the substance responsible for the increased urinary excretion of gonadotropin was not determined but it is believed to be of adenohypophyseal or adrenal origin. Since this phenomenon was observed to follow exogenously induced hyperadrenocorticalism the possibility suggested itself that it might also occur as a result of augmented endogenous adrenocortical activity. It is now well established that enhanced function of the adrenal cortex is an important part of the normal adaptive mechanism of the organism following stress or trauma. This is revealed in various studies dealing with the effects of injury on such indices of adrenocortical activity as the urinary excretion of neutral 17-ketosteroids (2) and corticoids (3), the level of circulating eosinophils (4, 5) and the electrolyte composition of thermal sweat (6). Patients subjected to elective surgical procedures constitute an ideal group in which the effects of increased adrenocortical function can be investigated. Johnson and his co-workers (6) interpret a major surgical procedure as an "alarming stimulus" resulting in increased adrenocortical activity during the first five to nine days after operation. The object of the present report is to record the results of assays for urinary gonadotropic activity in a group of subjects before and after surgical intervention. In a significant number of these patients the trauma of operation was followed by increased urinary gonadotropin titers.

MATERIAL AND METHODS

This study is based on observations in 18 patients who were subjected to a variety of surgical procedures under general anesthesia. None of this group showed any evidence of gonadal insufficiency or other abnormality which might produce or contribute to an abnormal urinary gonadotropin excretion. The operative procedures were of a major character in all instances except 2. The latter included a curettage of the uterus and an excision of a large keloid. Fourteen patients were female and 4 were males. The ages ranged from 13 to 60 years, with only 1 exceeding 50 years of age. This was a man aged 60 years.

Determinations of urinary gonadotropin were made before operation

(usually the preceding day), directly after the operation and on as many postoperative days as was technically feasible. In 5 cases the urine excreted during the first 24-hour postoperative period was collected during two consecutive 12-hour intervals and separately assayed. In several instances urine collections were not obtainable after the second or third postoperative day. In general, satisfactory urine specimens were available at 2-day to 4-day intervals for approximately one week.

The assay method employed was essentially that of Zondek (7) as modified by Klinefelter, Albright and Griswold (8), using immature female white mice weighing 6 to 9 grams. Certain additional technical variations included the use of a one-third aliquot of a 24-hour icebox-stored collection of urine instead of a 90-minute aliquot. Uterine enlargement, the criterion of a positive response, was considered to be present only if the uterine weight was 10 mg. or more. In our laboratory the uteri of control animals occasionally attain a weight of 8 or 9 mg., thus making the 7-mg. endpoint of Klinefelter *et al.* unsuitable. By this procedure the normal range of urinary gonadotropin excretion is between 5 and 45 mouse uterine units per twenty-four hours. Dialysis was not performed since no attempt was made to determine subnormal levels. The results of 70 assays with 204 dose-levels of urinary extract form the basis of this study. This represents an average of approximately 4 assays per patient, each assay usually being performed at 3 dose-levels. These figures are exclusive of numerous duplicate assays performed for the purpose of confirming results.

Not infrequently, groups of experimental animals failed to survive the period of observation. Since this often included control as well as treated animals it cannot be attributed entirely to urinary extract toxicity. The animal mortality accounts for the absence of data on certain crucial days, thus interrupting the continuity of the observed results. Furthermore, this is responsible for the fact that it was occasionally impossible to demonstrate normal or slightly elevated urinary gonadotropin titers. Nevertheless, it is apparent that these procedural impediments did not interfere significantly with the demonstration of well defined increases in urinary gonadotropic activity.

RESULTS

In 8 of the 18 patients an excessive quantity of urinary gonadotropin was demonstrated after operation (Table 1). In 2 of these 8 patients (Cases 7 and 8) the preoperative urinary level was not obtained. These individuals included a 39-year-old woman with multiple fibroids who was operated upon one month after her last menstrual period. Her previous menstrual history had been completely normal except for the last menses, which occurred one week early and were somewhat more prolonged and profuse than usual. The other subject was a 50-year-old man with a fracture of the femur at the site of a benign bone tumor. He presented nor-

mal genitalia and was married, although he had no children. From the history and clinical findings in these 2 patients there is no reason to suspect that their preoperative urinary gonadotropin excretion was above normal. Therefore, the demonstration of increased urinary gonadotropic activity after operation is regarded as genuine and significant. In the remaining 6 patients there was an unequivocal postoperative rise in the urinary gonadotropin content. The elevation of the urinary levels ranged from 60 to 360 mouse uterine units per twenty-four hours.

The 8 patients in whom an increased urinary gonadotropic activity was noted postoperatively had the following conditions: uterine fibroids 2, cholecystitis with cholelithiasis 1, gastric carcinoma 1, colonic carcinoma 1, fracture of the femur 1, menometrorrhagia (functional) 1, and mammary hypertrophy 1. Six were females ranging between 24 and 41 years of age. Two were males, aged 50 and 60 years. Five of the operations involved celiotomy with partial or total resection of abdominal viscera. The remaining surgical procedures included plastic revision of the mammae, the preparation and insertion of a bone graft and curettage of the uterus.

In each instance the increase in the titer of urinary gonadotropin appeared during the first four postoperative days. It was noted within the first twenty-four postoperative hours in 4 patients (Cases 1, 4, 5 and 6). In 2 of these 4, it occurred during the first 12-hour period (Cases 1 and 5), whereas in another (Case 6) its appearance was delayed until the second 12-hour interval. The increased titer was first noted during the third postoperative day in 3 patients (Cases 2, 3 and 8) and during the fourth postoperative day in another (Case 7). It is possible that if daily assays had been performed in these last 4 patients, increased urinary titers would have been observed earlier.

No precise statement can be made regarding the duration of the observed rise in urinary gonadotropic activity. This is due to animal mortality, occasional urinary extract toxicity and the absence of daily assays for adequate lengths of time. Nevertheless, the available data indicate that the increased urinary gonadotropin titer may drop sharply or disappear postoperatively (a) after the first twelve hours (Case 1), (b) after the first twenty-four hours (Case 4), or (c) after the fourth day (Case 7). On the other hand, the increased titer may persist (usually to a lesser extent) for as long as seven or eight days (Cases 2, 3 and 7) after operation.

A detailed analysis of the clinical records and ancillary laboratory data of the 18 patients comprising this study failed to yield an adequate explanation for the postoperative appearance of increased urinary gonadotropin excretion in some patients and not in others. There was no correlation between the presence or absence of this phenomenon and such factors as race, age or sex of the patient, the nature or duration of the disease, the type or duration of the surgical procedure, the type of anesthesia or the

TABLE 1. URINARY GONADOTROPIC ACTIVITY IN PATIENTS SUBJECTED TO SURGICAL PROCEDURES

Case No.	Sex	Age (yrs.)	Operation	Urinary gonadotropins (m.u.u. per 24 hrs.)*	Day of assay†
1	F	24	Cholecystectomy for cholecystitis and cholelithiasis	<45 240 <80 45–90	Preoperative. 1st 12 hrs. 2nd 12 hrs. 4
2	F	41	Mammoplasty	<45 <45 180 90–180	Preoperative. 1 3 7
3	F	36	Hysterectomy for multiple fibroids	<45 90–180 90–180	Preoperative. 3 7
4	M	60	Subtotal gastrectomy for carcinoma	22.5–45 90–180 30–90 45–90	Preoperative. 1 4 9
5	F	40	Partial colectomy for carcinoma	<33 60–80 <80 <60 90 45–90	Preoperative. 1st 12 hrs. 2nd 12 hrs. 2 4 6
6	F	41	Curettage for menometrorrhagia	<60 <40 80–120	Preoperative 1st 12 hrs. 2nd 12 hrs.
7	F	39	Hysterectomy and left salpingo-öophorectomy for multiple fibroids	30–60 360 90–180	1 4 8
8	M	50	Bone graft for fracture of femur	<90 <90 180	1 2 3

* Highest level tested, except where indicated by a negative result obtained at a higher level in order to establish a range.

† Counting from the day of operation.

postoperative fluid balance as estimated from the daily fluid intake and urinary volume. There was no instance of postoperative shock or hemorrhage.

DISCUSSION

The presence of increased gonadotropic activity in the urine of patients shortly after operation is consistent with the prior demonstration (1) of this urinary finding during cortisone and adrenocorticotropin therapy. The physiologic response common to both experimental groups is an increase in the amount of available adrenocortical hormones or their metabolites—in one instance of endogenous origin, and in the other instance of exogenous origin.

Investigations of the effects in man of surgical intervention on urinary gonadotropic activity have not been reported previously. In the experimental animal, Selye (9) describes ovarian atrophy as a result of surgical stress. This is attributed to a decrease in pituitary gonadotropin secretion consequent upon a simultaneous increase in the secretion of adrenocorticotropin. However, this hypothesis appears untenable in humans, at least in part, in the light of our observations. Furthermore, Selye's conclusions fail to find support in the observations of Glendening and her co-workers (10), who studied urinary gonadotropin excretion in human subjects immediately after the subjects had sustained burns. These investigators report a normal urinary content during the first twenty days. Subsequently, increases were found, which in some cases returned to normal. The clinical results described in the present report are derived from relatively short-term studies in humans. The observed rise in gonadotropic activity of the urine of patients treated with ACTH and cortisone is apparently time-limited, in that it often disappears or is decreased during the continuation of therapy (1). This would imply that the effect of certain types of stress on gonadotropic activity may be short-lived and that prolonged stress may eventually result in a decrease or disappearance of adenohypophyseal gonadotropic activity.

From the available data it is not possible to characterize the nature of the substance in the urine which is responsible for its increased gonadotropic activity. Potent urinary extracts were not tested in ovariectomized mice for the purpose of excluding a metrotropic rather than a gonadotropic agent. The negative results obtained by this procedure in the previously reported (1) group of patients treated with ACTH and cortisone suggest that an estrogen-like factor is not present. It is therefore probable that the substance has a true gonadotropic action and is derived either from the adenohypophysis or the adrenal cortex.

SUMMARY

1. Urinary gonadotropin assays were performed in a group of 18 patients who were subjected to surgical procedures under general anesthesia.

2. Excessive urinary gonadotropic activity was demonstrated in 8 of the 18 patients after operation. Its presence could not be correlated with the race, age or sex of the patient, the nature or duration of the disease, the type or duration of the surgical procedure, the type of anesthesia or the postoperative course or fluid balance.

3. The increased urinary gonadotropin titer appeared within the first four postoperative days in each of the 8 cases. In 4 patients it was noted during the first postoperative day.

4. The duration of the increased urinary level was variable. In one instance the increased titer dropped sharply after the first twelve postoperative hours, in another after twenty-four hours and in a third case after four days. Excessive excretion persisted in 2 patients for seven days, and in another for eight days.

5. The mechanism responsible for the postoperative appearance of excessive urinary gonadotropic activity is not known. It is probable that it is integrated with the adaptive response of the organism to stress.

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AN ATTEMPT TO ASSESS THE MAGNITUDE OF ENDOGENOUS ANDROGEN PRODUCTION IN A CASE OF THE ADRENOGENITAL SYNDROME*

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IT IS generally recognized that the steroidal hormones excreted in urine may provide unreliable qualitative and quantitative indices of endogenous hormone production. In recent years many procedures have been restored to in an effort to resolve this difficulty. One such consists in the calculation of endogenous hormone production from the percentage of the exogenous hormone recovered in the form of its metabolites in the urine as in the case of progesterone (1). A second procedure is based on the assumption that the amount of hormone required for complete replacement therapy in insufficiency states is equivalent to normal endogenous hormone production. Attempts have also been made to estimate endogenous hormone production from the hormonal content of the venous blood from a ductless gland, specifically, in the case of the adrenal (2).

It is the purpose of this study to describe another approach to this problem. This is based on the neutralization of an endogenous hormone by the administration of an appropriate oppositely acting hormone. This procedure is limited to the estimation of those hormones which produce mutually antagonistic biologic and biochemical effects.

An opportunity for such a study was provided by a classic case of adrenogenital virilism due to bilateral adrenal hyperplasia.

CASE HISTORY

M.F., *N.Y.H. 453646,* a 17-year-old white Jewish student entered the hospital with the chief complaint of amenorrhea and hirsutism. The hirsutism had appeared at the age

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of 4 and was progressive, eventually involving the face, extremities, anterior and posterior chest and lower abdomen. Axillary and pubic hair appeared at 7. Maximal skeletal growth was achieved at 12. Her weight had been constant for the past several years. The skin had always been coarse and oily and acne was present from the age of 3. Her voice had been low pitched and prone to crack for an unknown length of time. She had never menstruated and there had been no breast development. The clitoris had been noted to

FIG. 1. Patient M. F. Adrenogenital syndrome, prior to therapy.

be enlarged since the age of 8. She considered her strength to be better than average. Her interests were feminine.

In 1937, at the age of $8\frac{1}{2}$, she was seen by Dr. Murray B. Gordon and Dr. Bela Schick. We are indebted to Dr. Schick for the physical and laboratory examinations at that time. Her height was 57 inches and weight $95\frac{1}{2}$ pounds. She was a stocky masculine girl with hypertrichosis and a rough, coarse voice. Her eye grounds were unremarkable. The external genitalia showed a large penis-like clitoris with hypospadias. Vaginal examination under anesthesia revealed a vagina of normal size and depth with a smooth glistening mucosa and a small nipple-like projection about $\frac{1}{16}$ of an inch in diameter in the posterior vagina, which apparently had no canal. The uterus and adnexae could not be felt.

Laboratory findings in 1937 were as follows: serum chlorides 500 mg. per cent; BMR +11 per cent and +17 per cent. X-ray examination showed an advanced bone age, an unremarkable skull and normal intravenous pyelograms. The urine assayed at 5 units of "female sex hormone" and 3 units of "male sex hormone" per twenty-four hours and contained no prolan. No information was given as to the assay methods employed. Exploratory laparotomy was refused and the patient received a few injections of an estrogen.

At the time the present study was begun, in October 1946, the patient was 17 years old; her height was 155 cm., weight 55.98 Kg. and B.P. 128/80. She was a well nourished, stocky female with a masculine body configuration, male carrying angle and heavy musculature, as shown in Figure 1. There was marked hypertrichosis of the face, extremities, anterior and posterior chest, and abdomen. There was a moderate temporal regression of the scalp hair. Her voice was low pitched and flat. There was moderate acne over the upper back. Her skin was coarse and oily. No breast tissue was present and the nipples were small and slightly pigmented. The fundi were unremarkable and the visual fields normal. Indirect laryngoscopy revealed normal vocal cords. The clitoris was glans-like in appearance and structure and at least ten times the normal size. The labia were unremarkable, the vagina moist and "senile." The cervix and uterus were approximately one quarter to one half the normal size and the adnexae were not identified.

EXPERIMENTAL

A. *Methods*

The patient was studied on the Metabolism Ward of The Russell Sage Institute of Pathology over a period of nine months and throughout the study was maintained on a constant creatine-creatinine free diet with vitamin supplementation. The metabolic balances, collection and preparation of specimens and chemical analyses were carried out by the methods employed by this laboratory (3). Vaginal smears were stained with the Shorr single differential stain (4). In addition to chemical determinations of 17-ketosteroids, the urinary androgens were assayed for biologic activity by the capon comb growth method of Gallagher and Koch.[1] We are indebted to Dr. Benjamin Stimmel for the urinary estrogen assays, to Dr. Eleanor Venning for the estimation of glycogenic corticoids and to Dr. Nathan Talbot for the 11-oxysteroid determinations.

B. *Plan of study*

The study was designed to explore the feasibility of estimating androgen production from the amount of exogenous estrogen required to neutralize the effects of the endogenous androgens, utilizing appropriate biologic and biochemical criteria. The following indices were selected as potentially useful for this purpose:

1. The conversion of the atrophic vaginal smear, typical of the adreno-

[1] These assays were carried out by The Hormone Assay Laboratories, Inc., Chicago, Ill.

genital syndrome, to the follicular type. Previous studies from this laboratory have shown that a fairly quantitative relationship exists between the antagonistic effects of exogenous androgens and estrogens on the vaginal secretions. Thus, in the menopausal castrate, a dose of 50 mg. of testosterone propionate given by daily intramuscular injections was found to neutralize the estrogenic effects on the vaginal smear of 1 mg. (6, 000 RU) of estradiol benzoate given in the same manner (5).

2. The conversion of an atrophic to a proliferative endometrium. Whereas the neutralizing ratio for the above androgenic and estrogenic preparations is 50:1 by weight for the vaginal epithelium, the ratio for the endometrium has been found to be approximately 30:1 (6).

3. Induction of breast development.

4. Reduction in size of the clitoris.

5. Abolition of acne.

6. Reduction in rate of hair growth.

7. Restoration of normal skin structure. In contrast with their mutually antagonistic effects on the vaginal and endometrial epithelium, the combined action of androgens and estrogens appears to be necessary to establish the normal histologic pattern of the skin. This index was included because of its objective character, even though there was no information at hand as to what might be the optimal ratios for the repective hormones.

8. The elevation of the urinary citric acid excretion.[2] Androgens and estrogens exert opposite effects on the level of urinary citric acid excretion, estrogens increasing and androgens lowering the daily output of this urinary constituent (7).

9. The possible effects of estrogens in modifying the metabolic pattern of nitrogen, calcium, phosphorus and creatine metabolism.

RESULTS

A. *Laboratory data prior to the administration of estrogens*

The hematologic findings were as follows: hemoglobin 15.0 Gm., red blood cells 5.1 million per cu. mm., hematocrit 42, sedimentation rate 13 mm./hour, white blood cells 9,000 per cu. mm., with 28 per cent lymphocytes, 4 per cent monocytes, 3 per cent eosinophils, 50 per cent mature and 15 per cent immature polymorphonuclear cells. Urine analysis was unremarkable; maximal urine concentration was 1.024 and maximal dilution 1.002; phenolsulfonphthalein excretion was 59 per cent in two hours; and urea clearance, 81.5 per cent maximal and 115 per cent standard clearance.

An oral glucose tolerance test (1.75 Gm./Kg.) gave the following venous blood values: fasting 85, half hour 144, one hour 176, two hours 107, three hours 112, and four hours 57 mg. per cent. An intravenous glucose tolerance test (0.5 Gm./Kg. as a 20 per cent

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[2] We are indebted to our associate, Hertha H. Taussky, for carrying out the citric acid determinations.

glucose solution given in thirty minutes) gave blood values as follows: fasting 100, half hour 305, one hour 171, one and a half hours 108, two hours 68, two and a half hours 73, three and a half hours 76, and four and a half hours 81 mg. per cent. An insulin tolerance test (0.1 U./Kg., intravenously) showed the following venous blood sugar values: fasting 81, twenty minutes 58; thirty minutes 40, forty-five minutes 57, sixty minutes 67, ninety minutes 71, one hundred and twenty 87, two hundred and forty 86, and three hundred and sixty minutes 84 per cent. The basal metabolic rate was −13 per cent and the serum cholesterol level, 195 mg. per cent. The spontaneous creatinuria on a creatine-creatinine free diet ranged between 45 and 240 mg./twenty-four hours while the creatine tolerance was 93 per cent and the creatinine index (mg. per Kg. per twenty-four hrs.), 22. The initial blood chemistry values were as follows: serum calcium 10.6 mg. per cent, phosphorus 4.1 mg. and protein 7.5 Gm. per cent; alkaline phosphatase 2.1 Bodansky units; sodium 132 mEq./L., potassium 4.2 mEq., chlorides 107 mEq. and CO_2 combining power 24 millimols.

The urinary hormonal 24-hour excretions during the control period were as follows: 17-ketosteroids, 28.8–39.2 mg. (normal range in this laboratory, 8–14 mg.); androgens, 99 ± 23 I.U. (normal range, 23–34 I.U.; glycogenic corticoids, 62 units; 11-oxysteroids, 0.45 mg.; and follicle-stimulating hormone, less than 5 mouse units. Vaginal smears showed an atrophic mucosa.

Roentgenograms of the skull and chest, and intravenous pyelograms were unremarkable. Roentgenograms of the skeletal system showed all the epiphyses to be closed.

B. *The effects of estrogen administration*

The patient received two courses of estrogens,[3] during which the various indices previously cited were utilized. During the first study period estradiol benzoate was employed, in divided doses twice daily by the intramuscular route; during the second period, ethinyl estradiol by mouth in divided doses three times daily. In Figure 2 are summarized the observations made during the first study period of seventeen weeks.

The dosage of estradiol benzoate was progressively raised from the initial daily dosage of 5,000 RU to 180,000 RU per day intramuscularly. The first perceptible change attributable to this hormone was a fall in the urinary calcium excretion, which was reduced from the initial value of 126 mg. to 90 mg. per twenty-four hours before there were any detectable alterations in the vaginal secretions. This occurred at a dosage level of 15,000 RU per day. With increasing amounts of estrogen, the values fell to an average of 30 mg. per twenty-four hours (at 30,000 RU per day) and there was no further reduction with a subsequent increase in estrogen dosage up to 180,000 RU (30 mg.) of estradiol benzoate per day. The effects of estrogens on the overall calcium balance will be discussed subsequently.

The urinary citric acid values began to rise above the control values of 500 mg. per twenty-four hours with the daily administration of 10,000 RU, and continued to rise with increasing dosage of estrogens to a value of

[3] The estradiol benzoate (Progynon-B) and ethinyl estradiol (Estinyl) used in this study were generously supplied by The Schering Corporation, Bloomfield, N. J.

approximately 750 mg. per day, when the estrogen dosage had been raised to 30,000 RU per day. There was no further rise. These significant alterations in both of these indices occurred prior to any changes in the other measurements employed in this study.

The first alteration in vaginal cytologic pattern was observed at a dosage

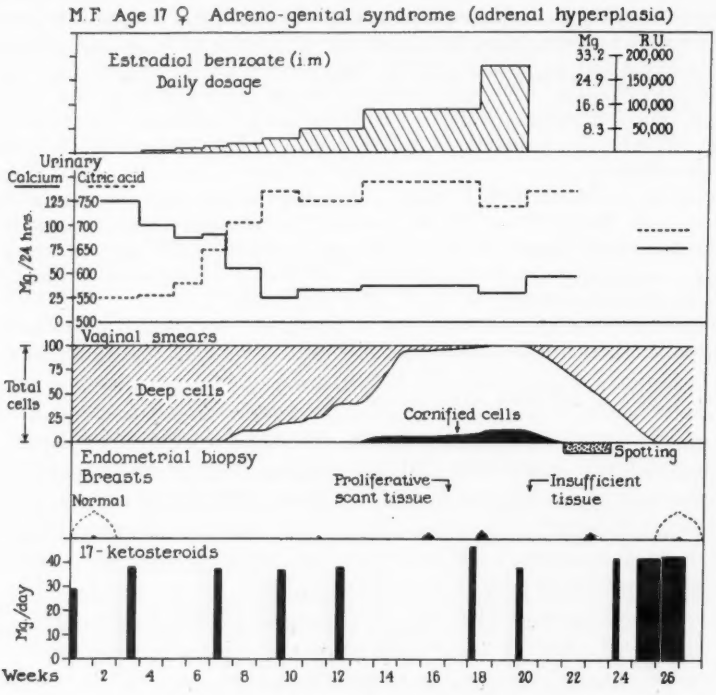

FIG. 2. Effects of estradiol benzoate therapy.

level of 20,000 RU per day. This consisted in a reduction of about 10 per cent in the number of deep cells. Thereafter, with increasing dosage, there was a progressive reduction of the percentage of deep cells; but not until the patient received 180,000 RU (30 mg.) per day were these cells completely eliminated from the vaginal smears. The first appearance of cornified cells occurred at a dosage level of 90,000 RU per day and with 180,000 RU per day cornification was present only to the extent of 10 to 12 per cent. The remaining cells were of the superficial type, with a progressive increase of the smaller nucleated variety at the higher dosage levels. On discontinuation of the estrogen there was a prompt disappearance of the cornified cells and a return of the typically atrophic smear.

Owing to the patient's initial reluctance, the first endometrial biopsy specimen was not obtained until the dosage level was 90,000 RU per day; it consisted of a scant amount of endometrial tissue with proliferative characteristics. A repetition of the endometrial biopsy when the patient was receiving 180,000 RU per day provided insufficient tissue for histologic evaluation. Beginning thirteen days after discontinuation of the estrogen, spotting appeared and persisted for approximately ten days.

The first detectable breast change appeared in the form of a subareolar node after the patient had received 90,000 RU of estrogen for approximately

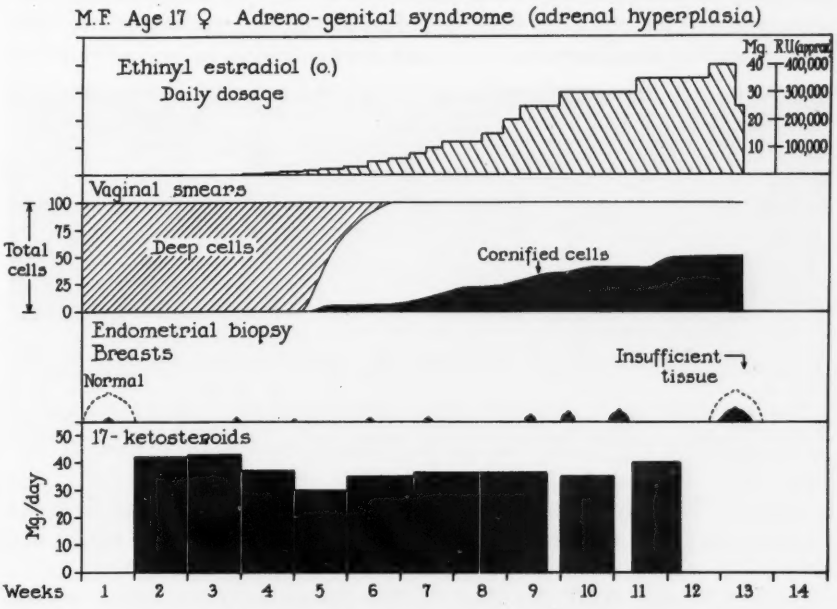

FIG. 3. Effects of ethinyl estradiol therapy.

three weeks. This was preceded by some tenderness and darkening of the nipple, first noted when receiving 30,000 RU. Some slight further enlargement occurred at 180,000 RU with a fairly rapid regression to the original state on discontinuation of therapy.

Acne was reduced at 50,000 RU, the rate of hair growth decreased at 90,000 RU and there was a perceptible reduction in the size of the clitoris with 180,000 RU per day.

17-Ketosteroid analyses showed no significant variations from control levels.

During these seventeen weeks the patient received in all, a total of

6,305,000 RU (1,050 mg.) of estradiol benzoate. At no time did the patient experience any intolerance to the natural estrogen.

The second experimental period was begun fifty-three days after estradiol benzoate had been discontinued (Fig. 3). Owing to the difficulty of administering larger doses of the natural estrogen intramuscularly the semi-synthetic estrogen, ethinyl estradiol, was employed by the oral route. Our bioassays of this estrogen in the human have shown that 1 mg. given orally is equivalent to approximately 10,000 RU, using estradiol benzoate intramuscularly as a standard (4). The ethinyl estradiol was given in progressively ascending doses to a maximum of 40 mg. per day, equivalent in the human to 400,000 RU of estradiol benzoate given intramuscularly. The total period of administration was nine weeks, during which she received

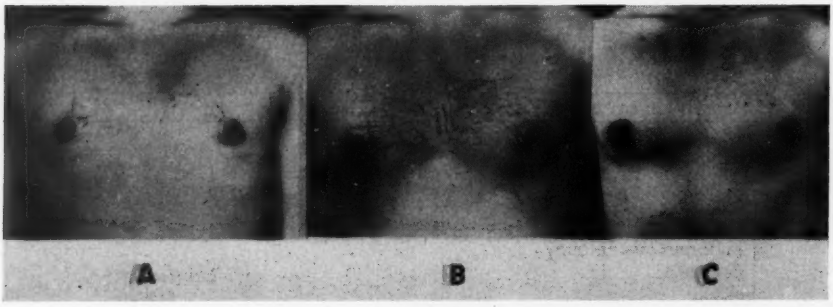

FIG. 4. Breasts prior to, and at the end of estrogen therapy. A. Prior to therapy. B. After 6,305,000 RU (1,050 mg.) of estradiol benzoate. C. After 1,178 mg. (11,780,000 RU) of ethinyl estradiol.

a total of 1,178 mg. or 11,780,000 RU. Except for vomiting on the first day of administration, this course of therapy was surprisingly free of the distress so frequently associated with the use of synthetic estrogens.

At the 1.5-mg. (15,000 RU) level the first reduction in deep cells occurred and these cells disappeared completely when the patient was receiving 5 mg. (50,000 RU). Cornified cells first began to appear at the 2 mg. (20,000 RU) dosage level and thereafter increased to a maximum of 50 per cent at a dosage of 40 mg. (400,000 RU) per day.

Subareolar nodes appeared in the breasts at the 20 mg. (200,000 RU) dosage level and the growth of breast tissue increased with ascending doses to the extent depicted in Figure 4.

Acne disappeared and the rate of hair growth was measurably decreased at the 25 mg. (250,000 RU) level. There was an appreciable reduction in the size of the clitoris at the 30 mg. (300,000 RU) dosage level.

The skin biopsy specimen obtained prior to the administration of es-

trogens was atrophic. The keratinized layer was thin and vacuolated and showed considerable desquamation. There were many prickle cells extending well into the basal zone. The corium was fibrous and avascular. The specimen was conspicuous for the absence of papillae and accessory structures such as sweat glands and hair follicles.

At the end of the period, during which the patient received a total of 1,178 mg. of ethinyl estradiol equivalent to 11,780,000 RU, the skin had acquired a much more normal histologic pattern. The keratinized layer was thicker but still vacuolated. Prickle cells were still present in the basal zone but in reduced numbers. The corium, although still fibrous, was more vascular. Papillae, sweat glands and hair follicles were present. The general picture was that of a skin with slight, if any, atrophic features but, on the other hand, with no signs of vigorous regeneration.

There was some slight reduction of uncertain significance in the excretion of 17-ketosteroids. During the control periods the average 17-ketosteroid excretion was 40.5 mg. per twenty-four hours, whereas the average excretion during therapy was 35.8 with a range of 30.0 to 39.2 mg. per twenty-four hours. Urinary citric acid and calcium determinations during this second experimental period could not be used as indices of estrogen influence owing to the fact, which was not appreciated until the study was completed, that calcium was present in the filler of the ethinyl estradiol tablets. As a result there was a progressive increase of considerable magnitude in the amount of calcium ingested, which deprived the urinary calcium and citric acid analyses of any specific significance.

At the end of the second period of estrogenic therapy the patient underwent an *exploratory laparotomy*. A vaginal examination under anesthesia showed the following: the clitoris was 4 cm. in length, with a well developed glans. The labia majora and minora were pigmented. The hymen was intact and the fourchette poorly developed. The cervix was small and nulliparous, and there was considerable retraction of the posterior fornix. The body of the uterus was anteflexed, 2.5 cm. long, and freely movable. The size of the uterus was considered to be within normal limits for an 18-year-old girl. The endometrial cavity permitted the entrance of a sound to a distance of 5 cm. A thorough curettage yielded a very small amount of atrophic appearing endometrium, which was insufficient for microscopic diagnosis. Both ovaries were felt to be small. They were 2 cm. in length and there was no evidence of old corpora albicantia or corpora lutea on their surfaces. Biopsies of the ovaries revealed a few primary follicles. The follicles had a degenerative type of epithelium and in some instances the epithelium was totally absent. There were several atretic follicles, as well as several small corpora albicantia. The fallopian tubes were normal in appear-

TABLE 1. METABOLIC DATA, SUBJECT M.F., SEVEN-DAY PERIODS. (ALL DATA GIVEN AS AVERAGE GM. PER DAY FOR THE PERIOD.)

Period No.	Medication	Nitrogen					Calcium					Phosphorus				
		Intake	Output			Balance	Intake	Output			Balance	Intake	Output			Balance
			Urine	Feces	Total			Urine	Feces	Total			Urine	Feces	Total	
1	E.B.* 50,000 RU	11.30	10.24	0.98	11.22	+0.08	1.398	0.039	1.232	1.271	+0.127	1.529	0.647	0.922	1.569	−0.040
2	E.B. 90,000 RU	11.30	10.79	0.91	11.70	−0.40	1.398	0.039	1.300	1.339	+0.059	1.529	0.744	0.914	1.658	−0.129
3	E.B. 90,000 RU	11.30	10.31	0.80	11.11	+0.19	1.398	0.029	1.000	1.029	+0.369	1.529	0.660	0.743	1.403	+0.126
4	E.B. 90,000 RU	11.35	10.25	1.12	11.37	−0.02	1.398	0.041	1.490	1.531	−0.133	1.529	0.645	1.093	1.738	−0.209
5	E.B. 180,000 RU	11.35	9.71	1.26	10.97	+0.38	1.398	0.031	1.379	1.410	−0.012	1.529	0.574	1.043	1.617	−0.088
6	None	11.35	9.57	1.00	10.57	+0.78	1.398	0.035	1.129	1.164	+0.234	1.529	0.642	0.861	1.503	+0.026
7	None	11.35	9.99	0.99	10.98	+0.37	1.398	0.057	1.179	1.236	+0.162	1.529	0.688	0.893	1.581	−0.052
8	None for 32 days	11.35	10.18	1.04	11.22	+0.13	1.398	0.080	0.893	0.973	+0.425	1.529	0.909	0.567	1.476	+0.053
9	None	11.35	9.56	0.99	10.55	+0.80	1.398	0.079	1.093	1.172	+0.266	1.529	0.778	0.714	1.492	+0.037
10	None	11.35	9.06	1.08	10.14	+1.21	1.398	0.069	1.307	1.376	+0.022	1.529	0.762	0.792	1.554	−0.025
11	E.E.† 30 mg.	11.30	9.68	0.94	10.62	−0.32	3.219	0.118	2.830	2.948	+0.271	1.528	0.346	1.064	1.410	+0.118
12‡	E.E. 35–40 mg.	11.30	10.15	2.03	12.18	−0.88	3.555	0.133	3.344	3.477	+0.078	1.528	0.345	1.183	1.528	±0.000

* E.B. = α-estradiol benzoate given intramuscularly per day.
† E.E. = Ethinyl estradiol given orally per day.
‡ Nine-day period.

ance. The adrenals were palpated and both were considered to be diffusely and equally enlarged.

Metabolic effects. The results of the metabolic balance studies are shown in Figure 5 and detailed in Table 1. Periods 8, 9, and 10 are regarded as

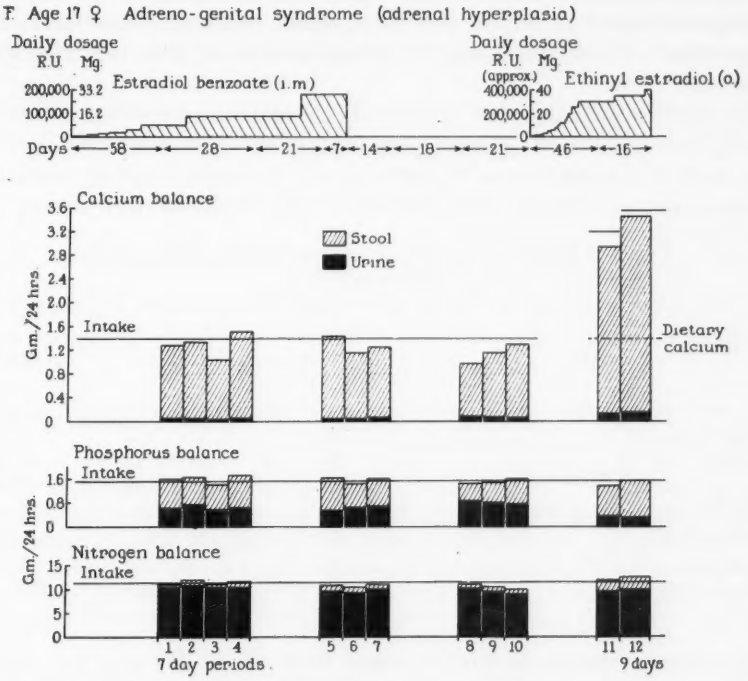

FIG. 5. Calcium, phosphorus and nitrogen balances.

representative of the basal state of the patient inasmuch as no estrogen had been administered for thirty-two days and the effects of the exogenous estrogen had disappeared at the time these balance periods were started. As mentioned previously, the increased calcium intake during periods 11 and 12 was inadvertent and was derived from the calcium filler of the ethinyl estradiol tablets. No significant or consistent changes were observed in the calcium, phosphorus and nitrogen balances during the administration of these large amounts of estrogen. The absence of any influence of the administered estrogens on the retention of any of these metabolic constituents suggests that the subject was storing at maximal capacity under the influence of her endogenous hormones. As noted before, reductions were observed in urinary calcium excretion during the period of adminis-

tration of estradiol benzoate; however, the overall calcium balance was unaltered. Indeed, the largest amounts of calcium retention occurred during several of the control periods.

The results of chemical analyses of blood taken during the course of study are summarized in Table 2. There were no significant or consistent changes observed in any of the blood constituents studied. Others have reported a fall in serum inorganic phosphorus in subjects receiving estrogens (8).

No significant changes were detected in creatine and creatinine excretion during the periods of estrogen administration. The creatinine index at the start of the study was 22, which is at the upper limit of normal for women, and a reflection of the patient's well developed musculature. The

TABLE 2. BLOOD CHEMISTRY DURING ESTROGEN ADMINISTRATION, IN SUBJECT M.F.

Serum	No. of determinations	Range	Average
Protein, Gm. %	23	6.21– 7.43	7.05
Calcium, mg. %	23	9.55–11.13	10.23
Phosphorus, mg. %	23	3.46– 5.43	3.99
Alkaline phosphatase, Bodansky U.	21	0.4 – 2.1	1.4
Sodium, mEq./L.	25	133–152	141.0
Potassium, mEq./L.	17	3.7 – 6.72	4.5
Cholesterol, mg. %	22	195–282	264.0

spontaneous creatinuria which ranged from 40 to 250 mg. per twenty-four hours on a creatine-creatinine free diet, was not affected by the estrogens.

Fate of the administered estrogen. Because of the failure of these very large amounts of estrogen to achieve complete feminization and to transform the atrophic state of the uterus and vagina to the typical estrogenic pattern, it seemed desirable to investigate the possibility that the subject was inactivating the exogenous estrogen at an abnormally high rate. Previous studies by Stimmel had provided information as to the amount of administered estrogen which could be recovered in the urine in the form of its metabolites after a standard dose, as well as the distribution of the excreted estrogen between estriol, estrone and estradiol fractions (9). A similar analysis was made by Dr. Stimmel of estrogen excretion in this patient at the time she was receiving 15 mg. (90,000 RU) of estradiol benzoate, representing 11 mg. of estradiol. The data are given in Table 3, along with the values he obtained in a female castrate following a single intra-

muscular injection of 1.6 mg. of estradiol benzoate, and in a normal pregnancy between the twentieth and twenty-eighth weeks. The normal range for the recovery of estrogens in the urine lies between 10 and 20 per cent, following a single injection of 1.6 mg. of estradiol benzoate. The patient under present study excreted 15.5 per cent of the estrogen administered, of which the estriol fraction constituted 48 per cent of the total as compared with 42 per cent in the female castrate. This contrasts with the high estriol

TABLE 3. DAILY EXCRETION (MG.) OF ADMINISTERED ESTROGEN IN SUBJECT M.F. COMPARED WITH THE EXCRETION IN A CASTRATED NORMAL FEMALE, AND DURING PREGNANCY*

	M.F.	Normal female†	Pregnancy, 20–28 weeks
Estrogen fraction	0.525	0.054	0.800–1.460
Estradiol fraction	0.334	0.011	0.270–0.900
Estriol fraction	0.810 (48%)	0.047 (42%)	0.390–7.000 (76%)
Total	1.669	0.112	1.460–9.360
Per cent of estrogen recovered	15.5	9.3 (normal range 10–20)	

* We are indebted to Dr. Benjamin Stimmel for these studies, as well as for the data on normal and pregnant women.

† Female castrate receiving a single injection of 1.6 mg. of estradiol benzoate. Patient M.F. received 90,000 RU (15 mg.) of estradiol benzoate intramuscularly daily during this study.

fraction of 76 per cent during pregnancy, which is currently attributed to an augmentation of the mechanisms for converting the large amounts of estrogen elaborated during pregnancy to this relatively inactive form. Apparently, the percentage of estradiol benzoate converted by this subject to the weakly estrogenic estriol was not abnormally high. Hence, the failure of these large amounts of estrogen to achieve complete feminization and overcome the effects of endogenous androgens on the vaginal mucosa and endometrium would not seem to be attributable to a gross abnormality in the metabolism of the exogenous estrogen, to judge from the urinary excretion pattern.

DISCUSSION

From the clinical standpoint, and judging from the level of 17-ketosteroid excretion in this subject, there was nothing to suggest the unusual magnitude of endogenous androgen production which became evident in the course of this study. The very large amount of estrogen required to

overcome the biologic effects of the endogenous androgens is unique, to judge from the literature. To bring about uterine bleeding, breast development, reduction in acne, in hirsutism and in the size of the clitoris, the maximal dosage hitherto reported to be required was 10 mg. of diethylstilbestrol daily, which is equivalent to approximately 15,000 RU.

Of the variety of indices used in this study only the vaginal smear provided data of a quantitative character. The information derived from the other observations, although interesting, could not be utilized for the quantitative assessment of endogenous androgen production. These latter observations will be discussed briefly before attempting to evaluate the information provided by the vaginal smear studies.

Among the metabolic data, a finding of interest was the creatinine index of 22, which is at the extreme upper limit of normal for women, the range being 14–22. Since the creatinine index is directly related to muscle mass, this high index, which falls within the normal range for men (20–28), is undoubtedly a reflection of the increased muscle mass resulting from the high androgen production. The reason for the moderate but persistent spontaneous creatinuria is obscure. However, the creatine tolerance following the ingestion of 1.32 Gm. of creatine was 93 per cent, an unusually high degree of retention for women.

The overall calcium, phosphorus and nitrogen balances were neither consistently nor significantly altered during the administration of estrogens. Despite the reduction in urinary calcium excretion, the total calcium balance was unaffected. Actually, calcium storage was higher during several of the control periods than during any of the periods in which the patient was receiving estrogens. Evidently, the storage capacity of the body with respect to these metabolic components was "saturated" under the influence of her endogenous hormones.

It is of some interest that the reduction of urinary calcium excretion was the first change to occur from the administration of estrogens. This was shortly followed by an increase in urinary citric acid. This latter change was consistent with previous observations in this laboratory on the action of estrogens and androgens in raising and lowering, respectively, the urinary citric acid output. Our renal functional studies indicate that changes in the excretion of citric acid are brought about principally by alterations in renal tubular resorption of this metabolite (10). With respect to the reduction in urinary calcium, also, a renal tubular effect is suggested by the absence of any significant changes from the control values in the level of serum calcium throughout the period of estrogen administration.

During the first course of therapy with estradiol benzoate, no significant alterations occurred in the 17-ketosteroid excretion. With the administra-

tion of ethinyl estradiol, the average 17-ketosteroid level was 35.8 mg. as compared with the control value of 40.5 mg. per twenty-four hours. It is uncertain what significance, if any, to attach to these changes as evidence for a suppressive action of estrogens on the adrenal cortex.

With regard to the other qualitative indices, the effects of estrogen were first noted upon the acne, next on the hirsutism, then on the breasts and lastly upon the size of the clitoris. It is of interest, in the light of the protein anabolic effects of androgens, that the skin was initially atrophic. The restoration of the skin towards a normal histologic pattern following the administration of estrogen reaffirms the necessity for the coparticipation of both estrogens and androgens in the maintenance of this structure.

It was anticipated that information of a more quantitative type would be provided by the reactions of the vaginal mucosa and endometrium. Unfortunately, endometrial biopsy specimens provided insufficient tissue for histologic studies. Failure of this structure to respond promptly to estrogens was not unexpected, judging from previous experiences with the delayed response of the endometrium in many subjects with long standing amenorrhea, particularly of the primary type.

We are on more solid ground with respect to the more sensitive vaginal mucosa, in which the prompt and specific response to estrogens can be anticipated, with rare exceptions, regardless of the duration of the amenorrhea. Certain information bearing on the quantitative aspects of this response is available, and can be used to estimate at least the order of magnitude of androgen production in this patient. On an average, a dose of about 3,000 RU of estrogen is required per day to produce full cornification in the menopausal or amenorrheic subject. In the patient under study, the percentage of cornified cells rose to 12 per cent with 180,000 RU of estradiol benzoate and did not exceed 50 per cent with 400,000 RU of ethinyl estradiol. Hence, it would seem safe to infer that the induction of full estrus would require more than 133 times the amount of estrogen that is sufficient for this purpose in the average amenorrheic or menopausal subject. How much more, is a matter of speculation. However, in the latter type of patient, as much more estrogen is usually needed to shift the smear from 50 per cent cornification to full estrus as is required to produce 50 per cent cornification from an atrophic state.

This resistance to the action of estrogens is attributable to the direct antagonism of the vaginal effects of estrogens by endogenous androgens. In the menopausal castrate, the ratio between exogenous androgens and estrogens at which the action of the latter on the vaginal mucosa is neutralized, is found to be approximately 50 to 1, by weight, for intramuscularly administered testosterone propionate and estradiol benzoate. Ex-

pressing the latter in rat units, 6,000 RU (1 mg.) is neutralized by 50 mg. of testosterone propionate. These values can be used in two ways to deal with the data given in Figures 2 and 3.

Neutralization of endogenous androgens by estrogens might be considered to have occurred when the atrophic characteristics of the vaginal smear had been abolished. During the administration of estradiol benzoate in the first study period, this took place at a dosage level of 180,000 RU or 30 mg. per day. This amount of estrogen would neutralize the effects of 1,500 mg. of testosterone propionate and hence, of the daily elaboration of an amount of endogenous androgen of equal biologic activity. During the ethinyl estradiol period, deep cells disappeared at a lower dosage level, 50,000 RU (5 mg.) per day; but the reasonable likelihood that the vaginal mucosa had not reverted to its original atrophic state after its recent stimulation by estradiol benzoate, makes the disappearance of deep cells during the second period of estrogen therapy less reliable as a measure of neutralization.

Estimation of endogenous androgen production by this criterion alone would be valid only if, at that point, full estrus were now achieved by additional estrogen in the amount (3,000 RU) which usually accomplishes this result in the menopausal castrate. Actually, in the ethinyl estradiol study period, the further addition of 350,000 RU (35 mg.) failed to induce estrus. Hence, the calculated daily amounts of endogenous androgens expressed in testosterone propionate equivalents (1,500 mg.) may be regarded as a minimum figure.

A second way in which the estrogen:androgen neutralizing ratio of 50 to 1 might be used to estimate endogenous androgen production would be to assume that the antagonistic effect of androgens on the vaginal mucosa was still operative and only partially overcome during the daily administration of 400,000 RU of ethinyl estradiol. This dosage corresponds to 67 mg. of estradiol benzoate, an amount of estrogen which would require for neutralization the daily production of androgen with a biologic activity equal to 67×50, or 3,350 mg. of testosterone propionate. On the basis of the average adequate replacement dosage of 10 mg. of testosterone propionate intramuscularly in the hypogonadal male, this would represent at least a 335-fold increase above the normal of endogenous androgen production in this subject.

It was conceivable that the exceedingly high estrogen requirements for vaginal cornification might have been due to the resistance of this end organ to estrogenic stimulation, a circumstance which, though rare, is occasionally met with. From the subsequent history of this patient, this would not seem to be the case. During cortisone therapy, 17-ketosteroid excretion fell to 5.5 mg. per twenty-four hours, and spontaneous menstruation oc-

curred in association with vaginal smears which were cornified to the extent of about 70 per cent during the ovulatory phase. This excellent response to her endogenous estrogens would argue for a normally sensitive vaginal mucosa.

The possibility was also explored that the resistance exhibited to the biologic effects of the administered estrogens might be attributable to their abnormally high inactivation by this subject. The findings of normal values for both the percentage of administered estrogen excreted, and the distribution of excreted estrogen between estradiol, estrone and estriol fractions makes this explanation unlikely.

It need hardly be emphasized that the calculated values given here for endogenous androgen production are offered only as approximations of the order of magnitude of hormone elaboration in this subject. The quantitative limitations of such calculations are appreciated, based as they are on a particular mode of hormone adminstration. Most hormones, given by continuous intravenous infusion, are much more effective than equal amounts given intramuscularly or orally, even in divided doses as in this study. Yet, estimations based on continuous intravenous infusion or pellet implantation are also subject to reservations as quantitative reflections of endogenous hormone production. For example, the daily absorption of 3 to 4 mg. of testosterone from pellets provides adequate replacement therapy in the hypogonadal male, whereas 17-ketosteroid excretion in the normal adult male is of the order of 15 mg. daily, a value which is only a fraction of the endogenous production, to judge from recovery experiments. The reasons have been given previously for regarding the calculated amounts of endogenous androgen production as representing minimal rather than maximal values; and it is at least permissible to conclude from these data and observations on other subjects with the adrenogenital syndrome receiving estrogens, that androgen production in this patient was of an exceedingly high order of magnitude and unpredictable either from the clinical picture or from the level of urinary 17-ketosteroid excretion.

SUMMARY AND CONCLUSIONS

An attempt has been made to assess the magnitude of endogenous androgen production in a classic case of the adrenogenital syndrome. The principle underlying this assessment was the utilization of exogenous estrogen to neutralize, and predominate over, the effects of the subject's endogenous androgens on a number of structures and metabolic processes upon which these hormones exert mutually antagonistic actions. A variety of indices were used including vaginal smears, endometrial and skin biopsies, changes in the breasts, clitoris, hair growth and acne, the levels of

urinary calcium and citric acid, as well as other metabolic processes. Of these criteria, only the vaginal smear provided quantitative data which could serve as a basis for calculating the order of magnitude of endogenous androgen production. Depending on the specific endpoint used in vaginal cytology, the minimal values for the daily androgen production of this subject were calculated to be equivalent to the biologic activity of from 1,500 to 2,500 mg. of testosterone propionate. These values are offered only as an approximation of the magnitude of endogenous androgen production.

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A BALANCE STUDY OF METHYLANDROSTENEDIOL IN A PATIENT WITH HYPOGONADISM

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THERE are several reports of the effect of methylandrostenediol on the nitrogen metabolism of patients. There is evidence that methylandrostenediol (MAD) has a definite nitrogen-retaining effect in the human being. Talbot *et al.* (1) observed in a young female with Addison's disease a daily nitrogen retention of 50 mg. per Kg. of body weight, when she was given a daily oral dose of 50 mg. of MAD. In this same patient on the same diet, methyltestosterone in a daily oral dose of 25 mg. resulted in a nitrogen retention of 100 mg. daily per Kg. of body weight. In 1 out of 3 dwarfs Wilkins (2) observed a protein anabolic action of an oral dose of 30 mg. of MAD daily. Thorn (3) obtained a positive effect on the nitrogen balance in 4 patients, with 25 mg. of MAD intramuscularly. Homburger *et al.*, (4) in comparing the nitrogen-retaining effect of MAD and testosterone, observed that 25 mg. of MAD daily for six days was about half as effective as the same amount of testosterone. No masculinizing effects were mentioned. Escher (5), Homburger (6), and several others found MAD therapy helpful when given in large doses to a number of patients suffering from mammary cancer. As a matter of fact, most of the data which are available in the literature were obtained in short experiments and only incomplete balance studies are described.

In the observations here reported, the effect of MAD on human metabolism was studied by measuring the nitrogen, phosphorus, potassium and sodium balances as well as the excretion of creatine, creatinine, and 17-ketosteroids in a patient during treatment with this steroid.

MATERIAL AND METHODS

The patient was a 20-year-old male whose height was 163 cm. and weight, 68 Kg. He had thin skin, a skeletal age of 15 years, female pubic hair distribution, a phallus only 4 cm. long, a small scrotum which was slightly wrinkled and a very small prostate; his testes could not be palpated. The FSH assay repeatedly gave negative results for 4 M.U. and the 17-ketosteroids were 6.6 mg. per twenty-four hours. A roentgenogram showed a normal sella turcica.

He was known to have received two courses of chorionic gonadotropin, five years and eighteen months, respectively, prior to the balance study. The exact amount was un-

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known, but the patient remarked that during the latter course erections were more frequent. His testes had not become palpable. It was not certain whether axillary or pubic hair growth was stimulated. The diagnosis was secondary hypogenitalism, cryptorchidism and secondary hypogonadism.

No treatment was given for four and a half months preceding the study. During the balance period the patient was alternately fed one of two diets equal in nitrogen content and calories. He was maintained on a strictly controlled regimen, in such a way that no appreciable variations in his daily bed rest and activities occurred.

Two daily urine specimens and four daily stool specimens were pooled and analyzed. the two diets were analyzed fortnightly. The estimations of the K, Na, P, and N content were performed after wet digestion of the material with concentrated sulphuric acid and Perhydrol (Merck). The nitrogen determinations were made with the Markham modification of the Kjeldahl method. Potassium and sodium were determined by flame photometry. The phosphorus content was estimated according to Briggs; the excretions of creatine and creatinine were measured by the method of Bonsnes *et al.* (7), and the urinary 17-ketosteroids by our own method. The patient was weighed every other day under standard conditions. The data are charted as proposed by Reifenstein *et al.* (8).

Plan of study. The patient was habituated to the diet and to the regimen for several days. The study was started with a control period of eight days, which was followed by a treatment period of twelve days and a final control period of twenty-eight days. During the treatment period a dose of 75 mg. of MAD[1] (microcrystalline suspension) daily was injected intramuscularly.

RESULTS

The data are charted in Figures 1 and 2. A definite retention of nitrogen, phosphorus, and potassium was observed during treatment with the drug. No significant effect on the sodium balance was seen. The excretion of creatinine was fairly constant. During therapy with MAD the excretion of creatine rose to 600 mg. daily. No change was found in the excretion of 17-ketosteroids. During MAD therapy the patient gained 1.7 Kg. in weight.

DISCUSSION

These data confirm the nitrogen-retaining effect of MAD. Since potassium and phosphorus are also retained, it is highly probable that new protoplasm is formed. However, a quantitative analysis reveals that the effect is more complicated. In Figure 2 the K, P, and N balance data are charted in such a way that the units of the scales are in agreement with the quantitative interrelationships of these elements in muscle protoplasm (Reifenstein *et al.* (8)). Evidently a much greater phosphorus retention occurred than could be calculated from the nitrogen retention. Although our data on the calcium balance were incomplete they suggested a slight

[1] Kindly supplied by N. V. Organon, Organon, Holland.

retention of calcium during MAD therapy, which was insufficient to explain this discrepancy.

The production of a marked creatinuria by MAD is a property in common with other steroids having a 17-methyl group in the molecule. Hober-

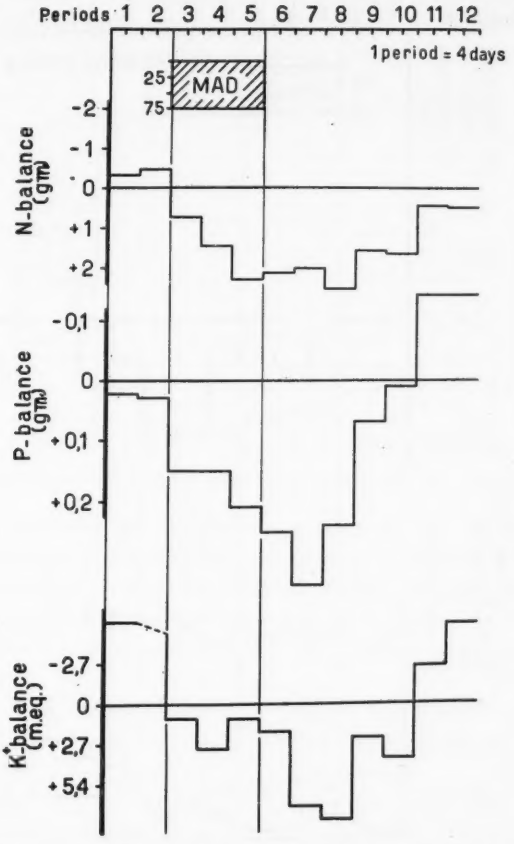

FIG. 1. A balance study of methylandrostenediol in a patient with hypogonadism.

man *et al.* (9) demonstrated in the case of methyltestosterone that the formation of creatine is stimulated in such a way that the capacity of muscle for storing creatine is insufficient, and the surplus is excreted. In muscle, creatine is stored partly in the free form, and partly as phosphate. It is probable that the increased creatine output which is observed under the influence of MAD therapy must be interpreted as an overflow of creatine from creatine-saturated muscle. From the discrepancy between the ob-

served and the theoretical phosphorus retention, it is possible to calculate to what extent creatine is stored as creatine phosphate in the muscle during treatment with MAD. Hardly any change in the excretion of neutral 17-ketosteroids was observed, indicating that the end products of MAD

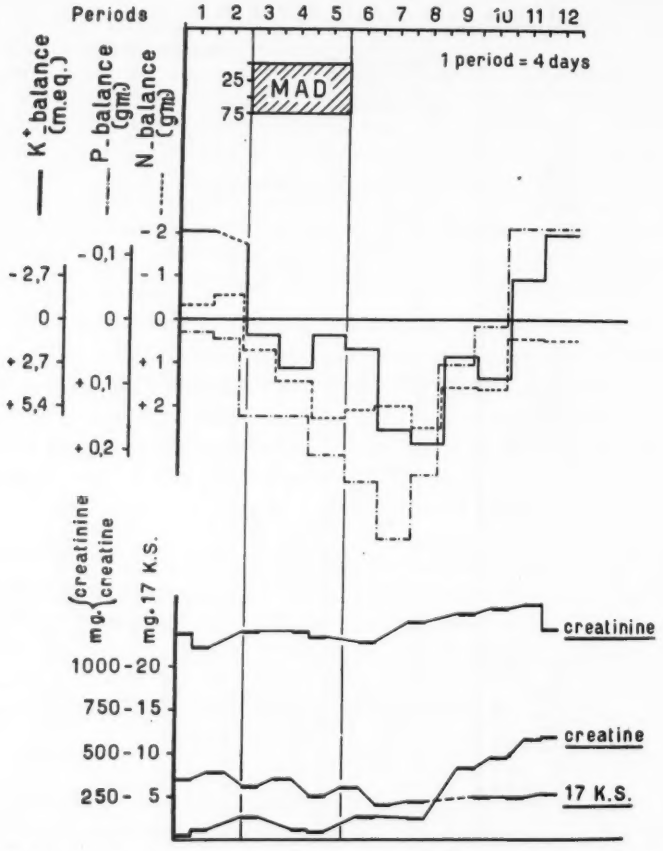

FIG. 2. Balances calculated in accordance with the method of Reifenstein *et al.* (8). Note the marked creatinuria and the discrepancy between the phosphorus retention and the nitrogen retention.

metabolism are of a non-16-ketosteroid nature. In the course of the experiment the patient retained 43.5 Gm. of N, equivalent to 272 Gm. of protein, or 1,320 Gm. of muscle protoplasm, in a period of thirty-two days. It is evident from our observations that the effects of the drug on the metabolism remain until twenty days after cessation of the injections. This may

be due to the fact that, by injecting the microcrystalline suspension, a depot was formed, which was only slowly resorbed by the organism.

In Figure 3 the actual weights and the theoretical weights as calculated from the nitrogen balance are charted. Obviously, in our experiment a discrepancy between the theoretical and the actual weight exists, the latter being higher than the former. According to Reifenstein (10) this indicates that extracellular water has been retained. In the testosterone-

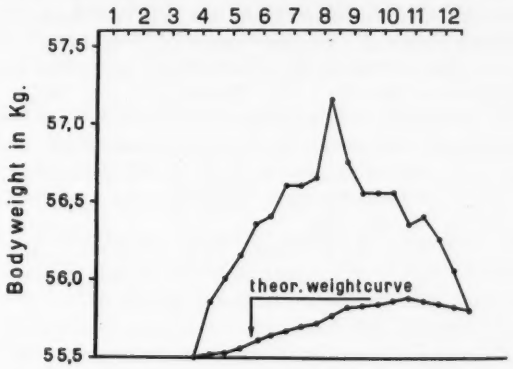

FIG. 3. Theoretical and actual weight curves during methylandrostenediol therapy.

treated mouse we obtained evidence for water retention in the *intra*cellular space of the kidney. We, therefore, should like to consider the possibility that this observation can be extended to other nitrogen-retaining compounds, and that MAD had this effect in our study. This hypothesis is favored by the fact that during MAD therapy no effect on the sodium balance was noted.

During the whole period of our investigation the clinical condition of the patient was excellent and no sign of an androgenic action of MAD (erections) was noted.

SUMMARY

1. A balance study is reported in a patient with hypogonadism who was treated with 75 mg. of methylandrostenediol (microcrystalline suspension) daily during twelve days.

2. Under the influence of the drug a retention of nitrogen (equivalent to 1,320 Gm. of muscle protoplasm), potassium and phosphorus was observed. No effect on the sodium balance was found.

3. The actual phosphorus retention was 590 mg. in excess of the theoretical phosphorus retention, as calculated from the nitrogen balance.

4. During the experiment, a definite creatinuria was observed. No effect on the urinary excretion of creatinine or 17-ketosteroids was seen.

5. A discrepancy was found between the actual and the theoretical weight curves, indicating that water was retained.

6. The clinical condition of the patient during the experiment was excellent. No sign of a masculinizing effect of methylandrostenediol was observed.

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THE EFFECTS OF SODIUM IODIDE, 6-PROPYL-THIOURACIL, AND 1-METHYL-2-MERCAP-TOIMIDAZOLE DURING RADIOIODINE THERAPY OF HYPERTHYROIDISM*†

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RADIOACTIVE iodine (I^{131}) is now well established as an agent in the therapy of Graves' disease (1–6). One disadvantage in its use has been a latent period of four to eight weeks between administration of the isotope and consequent clinical effect. The need for a study of the usefulness of antithyroid agents in obtaining earlier control over toxicity during this interval has been indicated from the work of others (2, 3, 7, 8). The present report deals with the results from sodium iodide, 6-propylthiouracil, and 1-methyl-2-mercaptoimidazole[1] given for a short period following I^{131} therapy.

METHODS

Eighty adult patients with unquestioned toxic diffuse goiter, selected at random, were treated by one of four methods: Group I received only radioiodine, I^{131}, in therapeutic doses; Group II, the isotope and sodium iodide; Group III, the isotope and 6-propylthiouracil; Group IV, the isotope and 1-methyl-2-mercaptoimidazole.

Radioiodine dosage was determined by one of us (S.C.W.) for all patients. Essentially, the degree of toxicity and size of the thyroid gland were the factors from which dosage was estimated. Radioiodine uptake was measured directly over the thyroid gland at twenty-four hours, as described elsewhere (1). The effective half-life of the I^{131} within the gland was determined from weekly measurements over the gland for four weeks following therapy (1, 9).

Antithyroid therapy, when used, was begun one week after radioiodine administration. It was continued for two subsequent weeks only, *i.e.*, to the

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[1] Obtained through the courtesy of Dr. D. C. Hines, Medical Director, Eli Lilly & Co.

third week after radioiodine was given. The patients were then followed without further treatment. Group II received 100 mg. of sodium iodide daily, Group III received 100 mg. of 6-propylthiouracil three times daily, and Group IV received 10 mg. of 1-methyl-2-mercaptoimidazole three times daily, each drug being given in addition to radioiodine.

Barker's modification of the Chaney method (10, 11) was employed for the determination of serum precipitable iodine, hereinafter referred to as SPI. The normal range for this method is from 3.5 to 7.5 gamma per cent. Basal metabolic rate determinations were made in the routine basal metabolism laboratory using the Benedict-Roth apparatus.

RESULTS

In Table 1 are summarized the data concerning the four patient groups. The values for basal metabolic rate, SPI and radioactive iodine uptake for

TABLE 1. PATIENT DISTRIBUTION AND PRETREATMENT LABORATORY VALUES FOR EACH THERAPEUTIC GROUP OF THE STUDY (see text)

Group	No. of patients	Females (% of total pts.)	Age (yrs.)		BMR (%)		SPI (γ%)		I^{131} uptake (%)	
			Average	Range	Average	Range	Average	Range	Average	Range
I	22	68	46	19–67	+36	+14–+76	12.0	5.3–20.6	46	27–59
II	25	88	46	26–61	+29	+ 3–+55	11.8	6.3–17.3	44	21–63
III	15	60	50	20–68	+31	+ 3–+69	13.2	8.2–18.9	50	16–65
IV	18	78	43	20–66	+29	+13–+50	11.9	6.6–16.3	44	20–66

each of the therapeutic groups show that the hyperthyroidism in all four was of about the same degree of severity. The average dose of radioiodine given in each group was similar (Table 2). The effective half-life of the

TABLE 2. AVERAGE DOSE OF RADIOIODINE AND THE EFFECTIVE HALF-LIFE OF THE ISOTOPE FOLLOWING I^{131} ADMINISTRATION

Group	No. of patients	I^{131} (dose in mc.)		Effective half-life in days			
				1st week		2nd week	
		Average	Range	Average	Range	Average	Range
I	22	4.0	2.5–6.5	5.3	3.5–8.0	5.1	2.5–7.0
II	25	4.0	2.5–6.5	5.1	3.0–7.0	4.9	3.0–7.0
III	15	4.0	2.5–6.0	5.7	2.5–8.0	5.2	2.5–8.0
IV	18	4.5	2.5–7.0	4.8	3.5–6.0	4.7	3.5–8.0

isotope within the gland after therapy was the same for each group, and did not appear to be altered by the antithyroid medication.

Statistical analysis[2] of SPI values from 76 patients before, and two weeks after radioiodine therapy is shown in Table 3. The mean SPI values for all

TABLE 3. STATISTICAL ANALYSIS OF SPI VALUES BEFORE, AND TWO WEEKS AFTER I^{131} THERAPY

Group	No. of patients	*SPI* (γ%)				*SPI* γ% decrease and S.D.		Statistical probability, %
		Initial		2 Weeks				
		Average	Range	Average	Range	Average	Range	
I	21	11.9	5.3–20.6	11.1	6.2–17.2	0.8±0.83	0.5–11.6	33.7
II	24	11.8	6.0–17.3	11.6	5.0–18.7	0.2±0.57	0.1– 5.8	72.6
III	14	13.6	8.2–18.9	12.8	5.7–21.2	0.8±0.71	0.1– 5.3	26.3
IV	17	11.9	6.6–16.3	8.3	2.8–16.6	3.6±0.76	0.0– 9.6	00.1

groups before I^{131} therapy were the same; two weeks later and one week after the initiation of treatment with the other agents, a decline in the average values of SPI from the original levels was seen in all patient groups. The mean decrease and standard deviation are shown, together with the probability in per cent that such a decrease could be due to chance. Only in Group IV, those receiving 1-methyl-2-mercaptoimidazole, was the decline in SPI statistically significant. The probability is less than one in a thousand that the observation could be due to chance.

SPI determinations were available in 56 patients four and eight weeks, as well as two weeks after radioiodine therapy (Table 4). Again, the afore-

TABLE 4. SPI VALUES BEFORE, AND AT TWO, FOUR AND EIGHT WEEKS AFTER I^{131} THERAPY

Group	No. of patients	SPI (γ%)							
		Initial		2 Weeks		4 Weeks		8 Weeks	
		Average	Range	Average	Range	Average	Range	Average	Range
I	15	12.1	5.3–20.6	10.9	4.9–17.2	11.0	6.2–17.1	7.8	4.1–20.6
II	15	11.8	6.0–17.3	11.1	5.0–18.7	10.3	6.6–17.7	8.1	1.8–12.8
III	12	13.3	8.2–18.9	12.6	5.7–21.2	10.7	4.7–14.3	9.6	2.6–16.0
IV	14	12.2	6.6–16.3	8.3	2.8–16.6	7.9	3.4–17.4	8.1	2.3–13.2

[2] The authors are indebted to Dr. John Fertig and Miss Ann Baranovsky for aid in the statistical analysis.

mentioned immediate fall in blood level at two weeks was apparent in the group treated with 1-methyl-2-mercaptoimidazole. However, the same ultimate values for SPI were seen at eight weeks in the remaining groups (I, II and III), as were found in Group IV.

The individual and average values for SPI are shown in Figures 1–4.

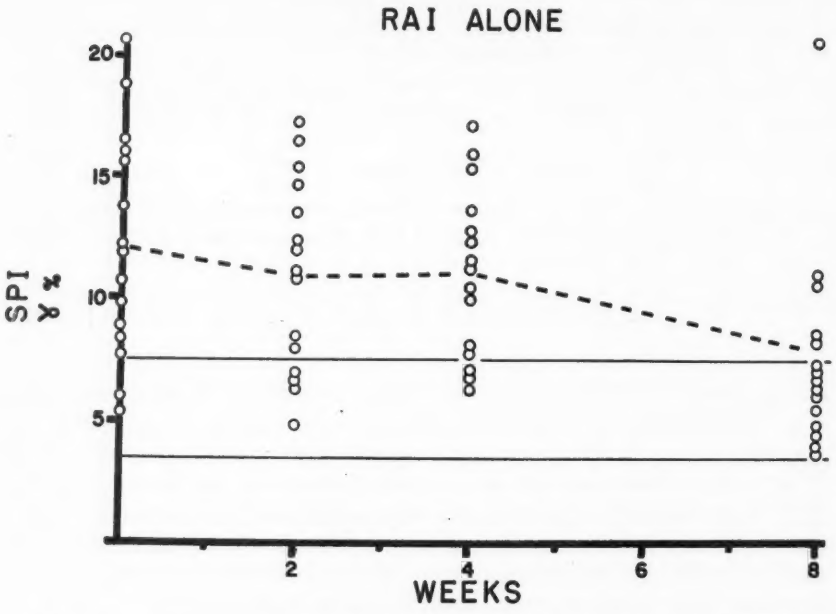

FIG. 1. Graphic representation of the SPI values from Group I, treated with radioiodine alone. (In this figure and in Figures 2–4, the circles are individual values; the broken line joins the mean for each time period and the horizontal lines across the graph enclose the normal range of serum iodine.)

The decline of the SPI in the first three groups was slow, and normal levels were not attained until about eight weeks after radioiodine was given. The SPI reached normal values in Group IV within two weeks after radioiodine was given and this level was found at four and eight weeks as well.

Three to four months after the administration of I^{131} SPI values were available for 54 patients. The average for 16 of these patients in Group I was 7.3 gamma per cent; for 20 patients in Group II, 8.0 gamma per cent; for 7 in Group III, 7.5 gamma per cent; and for 11 in Group IV, 6.7 gamma per cent.

The clinical response to the antithyroid therapy is worthy of note. The progress of the patients who received 6-propylthiouracil in addition to the

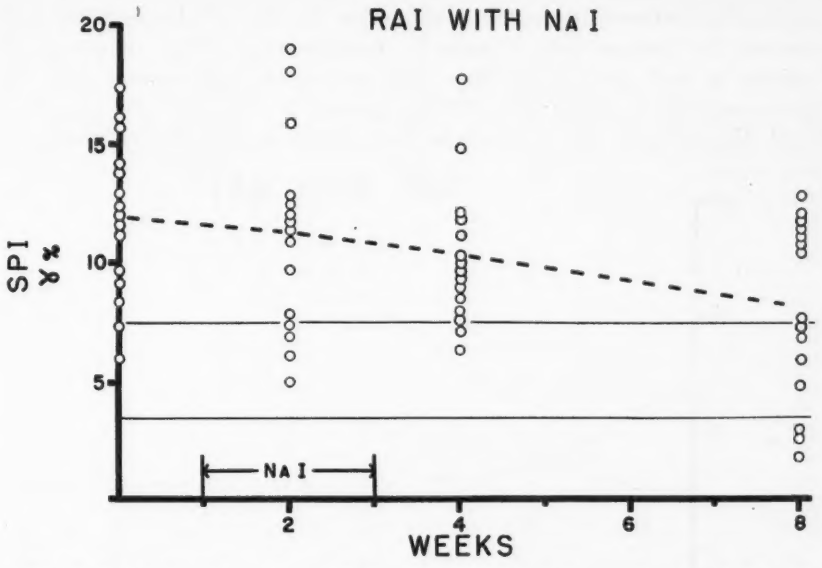

FIG. 2. SPI values from Group II, treated with radioiodine and sodium iodide. (In this figure and in Figures 3 and 4 the period of supplementary therapy is indicated in the lower left corner.)

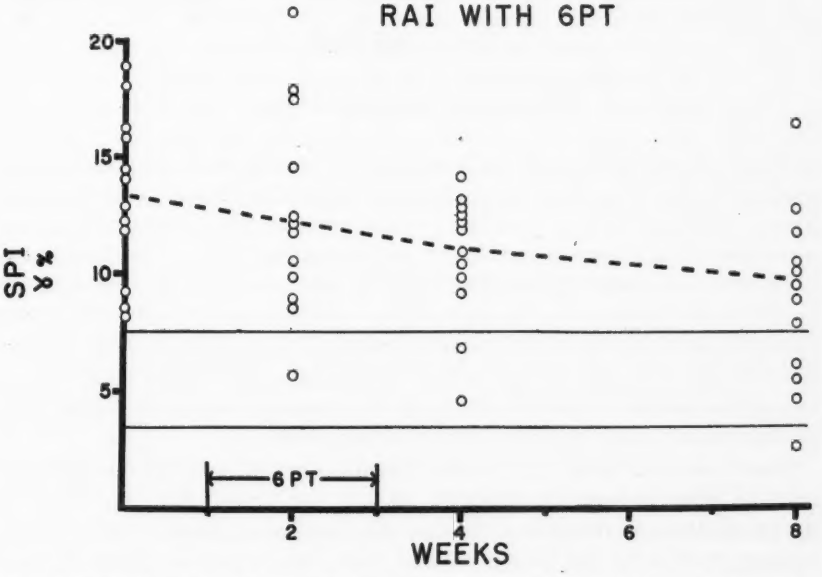

FIG. 3. SPI values from Group III, treated with radioiodine and 6-propylthiouracil.

radioactive iodine (Group III) was similar to that of the patients who received the isotope only (Group I). Amelioration of the symptoms of toxicity in both groups was slow, and subjective improvement over the six- to eight-week period after I^{131} was gradual in character. On the other hand, the patients who received sodium iodide or 1-methyl-2-mercapto-

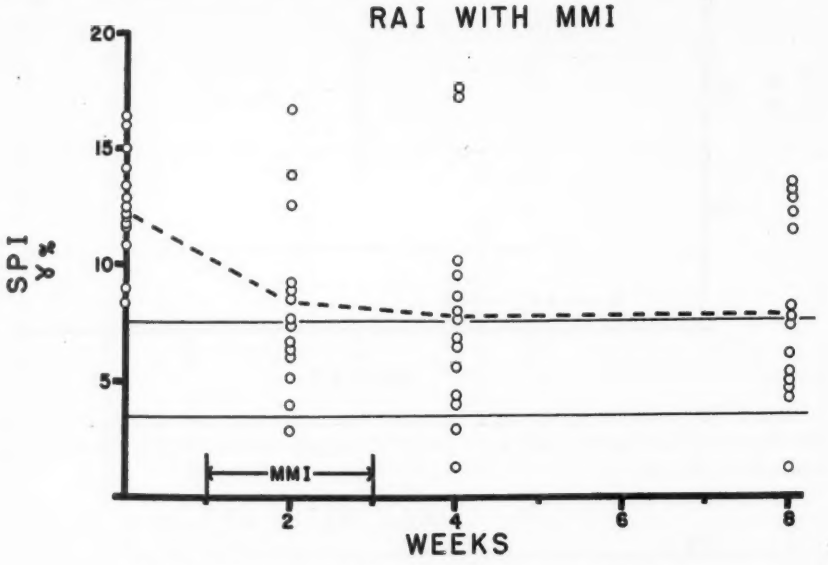

FIG. 4. SPI values from Group IV, treated with radioiodine and methyl-mercaptoimidazole.

imidazole were considerably improved by the second week after radioiodine administration (one week after the institution of antithyroid medication). Many of the patients in these two groups, who attained ultimate remission, maintained improvement after the antithyroid agents were discontinued.

In each therapeutic group there were a few patients who showed a rise in SPI level during drug therapy. Figure 5 shows the SPI values of 4 such patients, one from each of the four therapeutic categories. In each instance the SPI was seen to rise soon after I^{131} therapy, reaching a maximum at two to four weeks. Clinically, the patients appeared more toxic at this time, though none became critically ill as a consequence.

Basal metabolic rate values were determined initially and at one and three months after radioiodine therapy (except for Group III, in which the determinations were made at two months instead of three); Table 5 shows average results for the four groups at these time intervals. Table 6, simi-

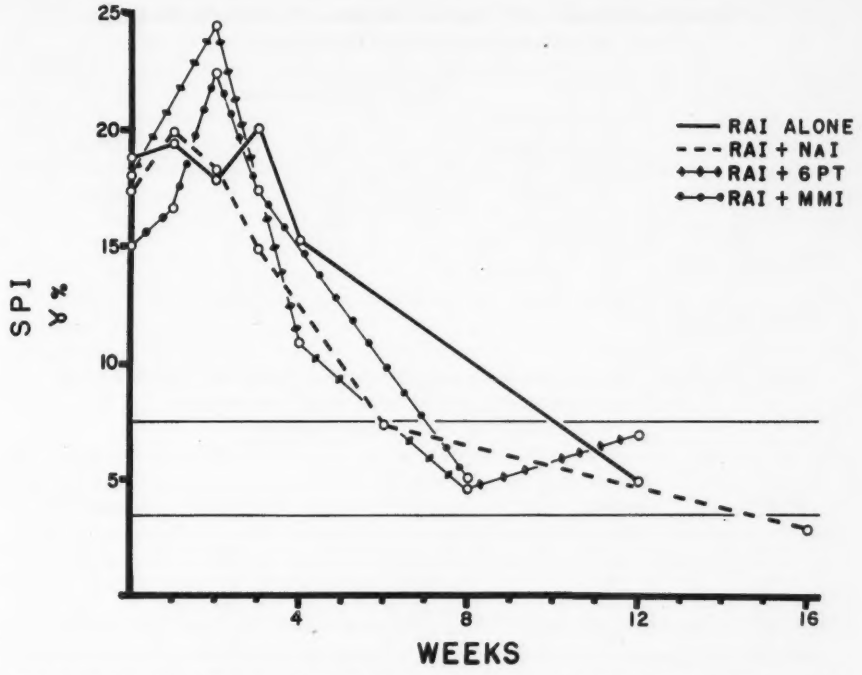

FIG. 5. Illustrative SPI values from the four patient groups, showing an elevation instead of a decline following I^{131} administration.

larly, shows average weights for the four groups at the same time intervals.

Table 7 summarizes the follow-up information concerning the four groups. With one exception all the patients have been followed for a minimum of seven months. The one not followed this long is known to have

TABLE 5. SUMMARY OF AVERAGE VALUES FOR BASAL METABOLIC RATE DETERMINED PRIOR TO RADIOIODINE THERAPY, AND ONE AND THREE MONTHS AFTER TREATMENT

Group	Average basal metabolic rate, %		
	Initial	1 Month	3 Months
I	+36	+14	+ 7
II	+29	+13	+ 1
III	+31	+17	+15*
IV	+29	+20	+ 9

* Two months.

TABLE 6. AVERAGE BODY WEIGHTS BEFORE, AND ONE AND THREE MONTHS AFTER RADIOIODINE THERAPY

Group	Body weight in pounds		
	Initial	1 Month	3 Months
I	133	137	136
II	133	136	147
III	129	133	137*
IV	137	145	140

* Two months.

TABLE 7. SUMMARY OF FOLLOW-UP DATA SHOWING NUMBER OF PATIENTS WITH ULTIMATE REMISSION, AND NUMBER WITH RECURRENCE SUBSEQUENT TO RADIOIODINE THERAPY

Group	No. of patients	Relief of toxicity*	Recurrence	Permanently hypothyroid
I	22	15 (58%)	7	2
II	25	13 (52%)	12	1
III	15	6 (40%)	9	0
IV	18	12 (66%)	6	1

* Includes patients rendered hypothyroid.

been in remission five months after therapy. The column headed "Relief of toxicity" includes both those patients rendered permanently hypothyroid and those in remission. The number of recurrences of toxicity is listed in the adjacent column. It is noteworthy that the remission rate from a single dose for Group I was similar to that consistently maintained in this clinic, about 67 per cent (9, 12). Groups II, III and IV showed varying remission rates, all lower than that of Group I. A critical examination of these results indicates that the difference in the percentage of remission found in Group I compared to that in Groups II and III approaches statistical significance. This does not hold when Groups I and IV are compared.

When the figures are reviewed for the larger series of patients treated in this clinic and not followed in such detail as the above, the same borderline significance obtains (13). Table 8 compares the results obtained from I^{131} supplemented as previously described with propylthiouracil, 1-methyl-2-mercaptoimidazole or sodium iodide with those in patients not receiving supplementary medication. Table 8 also takes into account the type of toxic goiter undergoing treatment, since toxic nodular goiter is said to be more resistant to I^{131} therapy (4). The suggestion is evident that more

TABLE 8. RESULTS OF I^{131} THERAPY IN PATIENTS RECEIVING, AND IN THOSE NOT RECEIVING, SUPPLEMENTARY DRUG THERAPY FOLLOWING RADIOIODINE ADMINISTRATION*

Diagnosis	No. of patients	No. of doses of I^{131}	Remission and hypothyroidism in group followed†		Total I^{131} dosage (mc.)	
			No. of patients	%	Av.	Range
I. Toxic diffuse and recurrent goiter						
A. No drugs	35	2	30	100	4.8	2–10
B. Drugs	70	3	59	97	8.7	3–67
II. Toxic nodular goiter						
A. No drugs	11	3	10	91	8.2	3–25
B. Drugs	30	3	24	90	11.8	3–30

* Modified from Werner, S. C.: *Med. Clin. North America.* May 1952, p. 623 (13).
† More than 6 months' follow-up period.

treatments and higher dosage are needed in those patients given supplementary drugs, but the wide spread of values again gives only borderline statistical significance to the results.

COMMENT

It has been shown that inorganic iodides and antithyroid drugs tend to inhibit the uptake of I^{131} by the thyroid gland (7, 14). Moreover, this effect may not wear off with reliable predictability. Williams has used antithyroid agents to achieve control over hyperthyroidism and has suggested their discontinuance for several days when therapeutic radioiodine administration is desired. This program has been designed to avoid the six- to eight-week lag before the radiation effect from I^{131} becomes evident. However, the action of iodides and antithyroid agents is not reliably terminated within a few days after cessation of their use. As a consequence, Williams' program has not been consistently successful in the experience of this clinic. The gland has too frequently not taken up radioiodine, and inadequate or no therapy has resulted, with considerable loss of time and irregularity of I^{131} dosage.

Accordingly, antithyroid drug and iodine have been given in this clinic after I^{131} therapy, in the period before the radiation effect has become manifest. An interval of one week was chosen between I^{131} therapy and subsequent drug administration, to establish the effective half-life of the

isotope within the thyroid before and after the provision of additional drug. The added medication was continued for two weeks only, to provide limited control over toxicity, which would be dissipated by the time a therapeutic effect of the isotope might be expected. Hertz and Roberts (2) have given potassium iodide following radioiodine administration, commencing twenty-four hours to several weeks after isotope therapy. They continued the iodide medication for from two to four months, making if difficult to establish which agent caused improvement during this period. The administration of iodides or antithyroid agents within the first twenty-four hours after I^{131} may result in inhibition of uptake of the isotope or of subsequent organic binding.

The present study reveals that 1-methyl-2-mercaptoimidazole is the most effective of the agents usually used in controlling thyrotoxicity, as judged by the rate of fall of SPI and improvement of the clinical picture. This corresponds with the experience of others as to the efficacy of this material (15, 16, 17). The short-term use of 6-propylthiouracil apparently did not affect the SPI significantly in this experiment and there was no definite clinical change. The patients receiving sodium iodide were improved clinically but revealed no concomitant reduction in SPI. This may be due to the fact that inorganic iodide administration may cause a "spurious" elevation of the SPI (18, 19, 20).

The results from this study tend to suggest that the use of antithyroid drugs and iodine shortly after I^{131} therapy may result in a somewhat increased resistance to radiation effect from I^{131}. There is a tendency to require more doses of I^{131}, or a higher total dosage of the isotope, before remission is achieved. However, this difference in result from that in patients receiving no medication beyond the isotope does not achieve more than borderline statistical significance and may not hold up when a larger series has been studied. At present it appears that the routine use of iodides or antithyroid agents is feasible but is probably not essential, except for patients in whom early control over toxicity is urgent. If such drugs are used, a compensatory increase in I^{131} dosage is probably in order. The mechanism for increased resistance to internal radiation effect as a consequence of iodides or antithyroid agents, if such an effect is confirmed, remains to be elucidated.

It is of some interest that the effective half-life of the isotope within the thyroid has been uninfluenced by iodides or antithyroid drugs. This is in contrast to the report of Goldsmith and co-workers (21) who found that in several instances there was a definite decrease of the biologic half-life in patients with thyrotoxicosis receiving 1-methyl-2-mercaptoimidazole and tracer doses of I^{131}. This discrepancy would tend to indicate that the therapeutic doses of radioactive iodine used in the present study, as op-

posed to the tracer doses employed by the Boston group, had sufficient injurious effect on the thyroid cells to abolish the secretory responsiveness noted in the other study.

Another point of interest is the failure of a sharp rise in SPI level, noted in several patients of the present series, to be accompanied by thyroid "crisis" or "storm." The behavior of the SPI has been studied previously after x-ray, surgical and radioactive iodine therapies for toxic goiter (22, 23, 24) and similar increases have been noted occasionally—again without "crisis." This agrees with the clinical impression that thyroid "crisis" is not the consequence of an exacerbation of the disease but rather is the end result of long-standing and neglected disease, regardless of the degree of thyrotoxicosis (25). However, there are not enough data available to settle this point.

SUMMARY

1. Four groups of thyrotoxic patients have been treated with therapeutic doses of radioactive iodine. Three of the groups have been given, in addition, either sodium iodide, 6-propylthiouracil, or 1-methyl-2-mercaptoimidazole for a two-week period commencing one week after administration of radioiodine.

2. An early and significant reduction in the serum precipitable iodine level has been induced by 1-methyl-2-mercaptoimidazole but not by sodium iodide or propylthiouracil.

3. Clinical improvement has been noted with sodium iodide and with 1-methyl-2-mercaptoimidazole administration several weeks before an effect from radiation with radioiodine could have been expected. No such benefit resulted from 6-propylthiouracil.

4. The percentage of patients achieving remission from a single dose of isotope may have been reduced, and the total dosage of I^{131} required to induce remission increased, as a consequence of the supplementary medication. Only borderline statistical significance can be attributed to this finding.

5. The effective half-life of the isotope within the thyroid has been unaltered by the iodides or antithyroid drugs used in this study despite the different mechanisms of action upon the gland.

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THE EFFECT OF GLUCOSE ON THE CIRCULATING EOSINOPHILS

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THERE are various means of investigating the functional activities of the adrenal glands. Among these, the enumeration of the circulating eosinophil cells, before and after the administration of adrenalin, has been very popular because of its simplicity.

It is an old belief that "stress" produces eosinopenia and lymphopenia (1). Recently Dalton and Selye (2) have shown the eosinopenic and lymphopenic effect of the alarm reaction. ACTH and corticoids also have an eosinopenic and lymphopenic effect (3, 4).

As early as 1908 Babes and Jonescu showed the hypertrophic effect of adrenalin on the adrenal glands (5). Later Vogt found that, after the administration of adrenalin, the amount of cortical hormones increased (6). Long and Fry found that in hypophysectomized animals, adrenalin had no such effect (7). Because of this, Long and Vogt believed that the effect of adrenalin was mediated by the pituitary gland.

It has recently been recorded that the ingestion of glucose decreases the number of circulating lymphocytes and eosinophils. Jailer (8) observed a decrease of circulating lymphocytes after oral administration of 100 grams of glucose. In Cushing's disease and in diabetics this decrease was more severe than in normal subjects, whereas in Simmonds' and in Addison's diseases it was less severe. Recant *et al.* reported that the administration of glucose decreased the number of the circulating eosinophil cells (1).

The purpose of this paper is to investigate the relationship between the depletion of the circulating eosinophils and the variations in the blood sugar level.

METHODS AND MATERIALS

This investigation has been carried out on 10 normal subjects, 10 diabetics and 3 patients with Addison's disease.

Before and after the oral administration of 50 Gm. of glucose, the blood sugar level was determined every thirty minutes during three and a half hours. The eosinophils were simultaneously counted during four hours. The Thorn test was performed on another day on the same patients.

The normal subjects were given glucose in divided doses, the first dose being 50 Gm. and the others being 10 Gm. every thirty minutes. The blood

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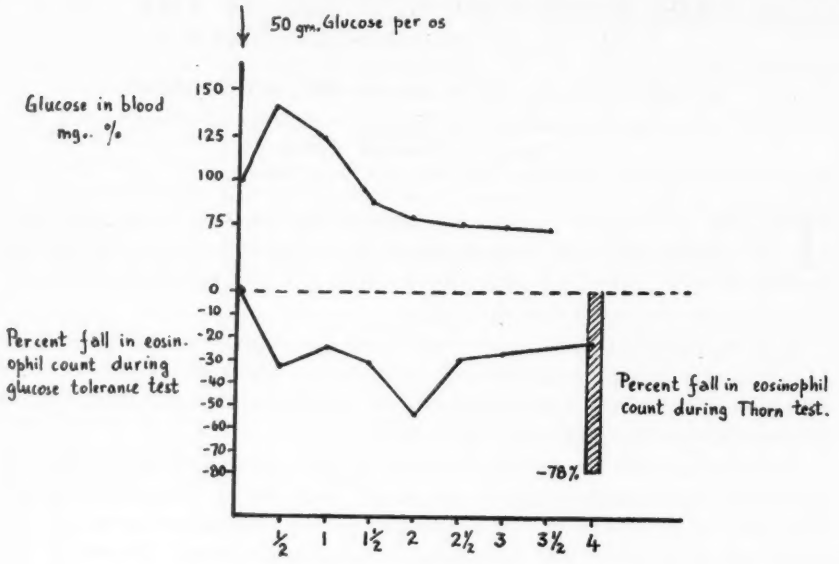

FIG. 1. Average of curves in 10 normal subjects.

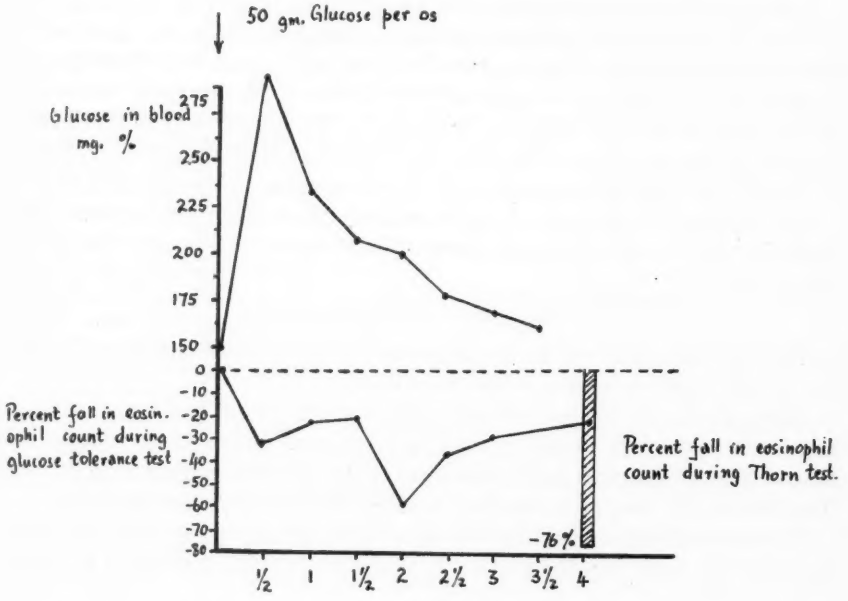

FIG. 2. Average of curves in 10 diabetic patients.

sugar and circulating eosinophil values were obtained as just described.

Blood sugar was determined, using the method described by Hagedorn-Jensen (9). Eosinophils were counted using the method described by Recant *et al.* (1).

RESULTS

1. In the normal subjects the circulating eosinophil cells decreased twice during four hours, under the effect of glucose. The first decrease coincided with the highest level of blood sugar (about thirty minutes after the administration of glucose) and the second decrease took place after the blood sugar had fallen to below the initial level (about two hours after the administration of glucose). The first decrease of the eosinophils was less than 50 per cent and the second decrease was more than 50 per cent (Fig. 1).

2. In the diabetic patients also, the eosinophil cells showed two periods of decrease, slightly more marked than those of the normal subjects (Fig. 2).

3. In the Addisonian patients the first decrease of eosinophil cells was the same as in normal patients but the second decrease was less than 50 per cent (Fig. 3).

4. During the administration of glucose in divided doses, the first decrease of eosinophil cells occurred as usual, but there was no other decrease throughout the duration of the high blood sugar level (Fig. 4).

DISCUSSION

The fact that the second decrease of eosinophils was less than 50 per cent in Addison's disease, whereas it was over 50 per cent in normal and in diabetic subjects, would indicate that normal adrenal glands are necessary for the latter effect.

Vogt has shown by perfusion of the isolated adrenal glands that variations in the amount of glucose in the perfused liquid have no effect on the corticoid production of the adrenal glands (10). Thus, the effect of glucose seems to be mediated by the pituitary gland. Judging by the effects noted on the circulating eosinophils, it is the decrease and not the increase in the blood sugar level that stimulates the pituitary-adrenal system. During the administration of glucose in divided doses, the high blood sugar level was maintained, and consequently there was no decrease in circulating eosinophils. This finding agrees with that of Vogt. She found that after demedullation of both adrenal glands the administration of insulin increased the production of corticoids, but that if the hypoglycemic effect of insulin were diminished by the simultaneous administration of glucose, the corticoid production of the adrenal glands fell. As a result, Vogt concluded that it was the decrease in the blood sugar level that stimulated the pituitary-adrenal system (11).

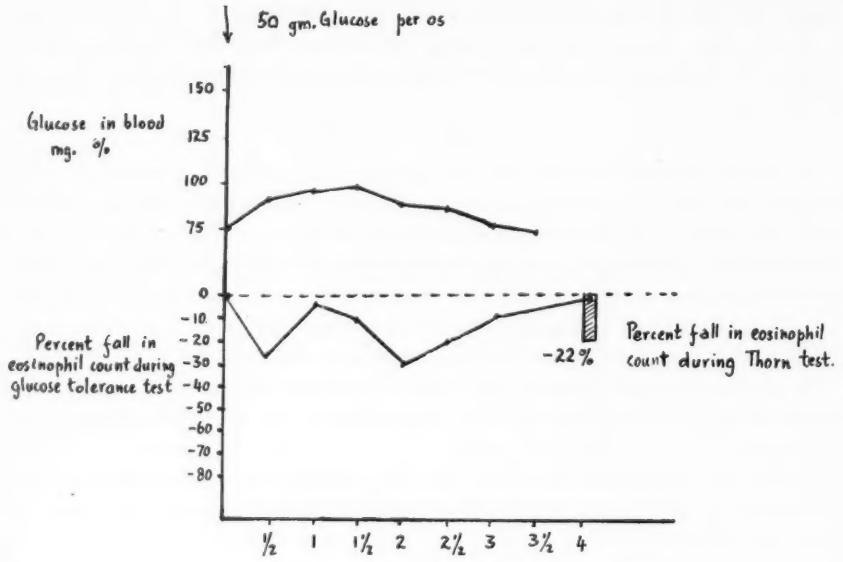

FIG. 3. Average of curves in 3 Addisonian patients.

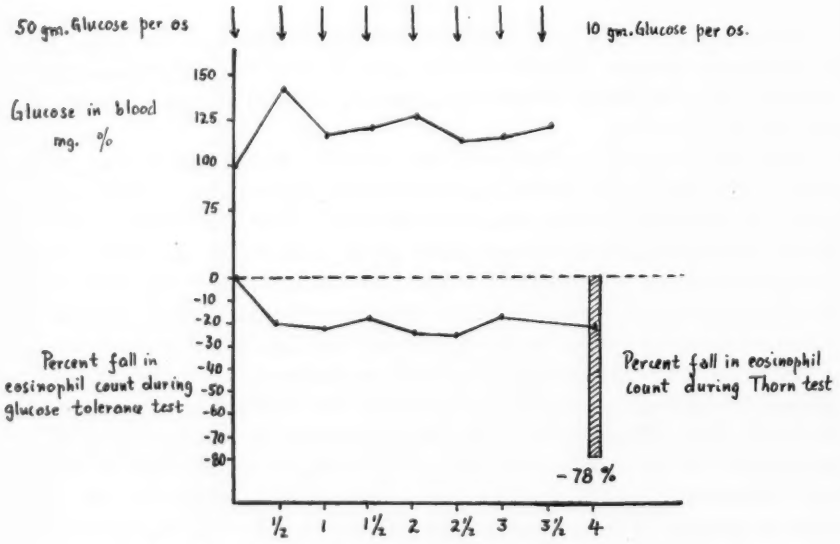

FIG. 4. Average of curves in 5 normal subjects during administration of glucose in divided doses.

Since the first phase of the decrease in eosinophils is not associated with a decrease in the blood sugar level, this phase would seem to bear no relation to the pituitary-adrenal system; its mechanism is unknown. On the other hand, the second phase of the decrease in eosinophils is associated with a decrease in the blood sugar to a level as low or lower than that obtained initially; this phase may be mediated by the pituitary-adrenal system.

SUMMARY

1. The effect of glucose on the circulating eosinophils has been studied.

2. Glucose caused a decrease in the circulating eosinophils, occurring in two phases; the first decrease took place thirty minutes and the second, two hours after the administration of glucose.

3. The mechanism of the first decrease is unknown but that of the second decrease may be mediated by the pituitary-adrenal system.

4. It is not the elevation of the blood sugar level, but rather the decrease to the initial level or below, that stimulates the pituitary-adrenal system.

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Letter to the Editor

A CASE OF STRUMA CIBARIA

To the Editor:

Since Chesney in 1928 induced goiter in rabbits by feeding cabbage, and Hercus and Purves in 1936 by feeding Brassica seed (1), a considerable amount of work has been reported regarding the goitrogenous action of vegetable food stuffs. The subject has been extensively reviewed by Greer (1) and also by Fertman and Curtis (2). Whereas there is broad evidence that certain vegetables contain goitrogens, very few instances of human goiter induced by excessive intake of such vegetables have been reported. Means has coined the term "struma cibaria" for this type of goiter (3). Himsworth (4) observed goiter in monks in a Belgian monastery, who, during World War II, were forced to use rape seed oil in large amounts. Rawson (5) has noted the case of a woman, whose goiter appeared to be caused by ingestion of large amounts of cabbage. Because of the paucity of such observations, the following case is reported:

Mrs. A. S., a 56-year-old Negress, came to the Endocrine Clinic of Jefferson Medical College Hospital in October 1950 because of swelling of her neck of five years' duration. She complained of somnolence and forgetfulness, but on close questioning these symptoms were of mild degree. Her past history included influenza in 1918, and uterine fibroids, for which she was operated upon in 1935. Her menses had been scanty up to the time of the hysterectomy. She had never been pregnant.

She was born in the coastal region of North Carolina, moved to Philadelphia as a child and had lived there ever since. There was no known incidence of goiter in her family.

Both she and her husband were unemployed and lived on a small relief allowance. Because of her limited means and, judging by her obesity, a rather unlimited appetite, she was forced to purchase the cheapest food available. She stated that for the preceding five years she had eaten rutabaga turnips often twice daily, ordinary turnips four to five times weekly, cabbage three times weekly, and parsnips "frequently."

Physical examination revealed a well developed obese woman. The skin was of normal texture, moisture and temperature. Hair texture and distribution was normal except for minimal facial hirsutism. There was no puffiness of her face, nor any huskiness of her voice. Funduscopic examination showed arteriosclerotic changes, Grade 2. The thyroid gland was considerably enlarged to about three times normal size; the consistency was quite soft. Close to the left lower pole a nodule, measuring about 4 cm. in diameter, was palpable; it was considerably harder than the remainder of the gland. The blood pressure was 120/80. The pulse rate was 84. She weighed 242 pounds.

Roentgenograms of the chest showed a minimal substernal extension of the thyroid with displacement of the trachea to the right.

The patient received a tracer dose of I^{131} (100 microcuries). After twenty-four hours the uptake by the thyroid was 8 per cent.

A tentative diagnosis of struma cibaria was made and the patient was instructed to refrain from the goitrogenic vegetables. She returned after one month and stated that she had conscientiously adhered to the diet prescriptions. The goiter had regressed almost completely, with the exception of the nodule in the left lobe, which had diminished in size and now measured 2 cm. in diameter. At this time her BMR was −1 per cent and the uptake of I^{131} was 4 per cent (twenty-four hours).

She continued to abstain from the goitrogenous vegetables. In May 1951 the BMR was −6 per cent and the uptake of I^{131} was 19 per cent. There appeared to be no appreciable change in her symptoms, though she stated at that time that she was less tired and less forgetful. Since these symptoms never had loomed very large in the picture, the alleged "improvement" was questionable. In November 1951 the uptake of I^{131} was 16 per cent. There was no recurrence of the goiter and the nodule remained unchanged.

The prompt reduction in size of this patient's goiter upon withdrawal of goitrogenous vegetables makes it appear very likely that the latter had been the cause of this goiter. Whether the nodule represents a secondary change in the hyperplastic gland, or whether the food-induced hyperplasia developed in a gland already bearing a nodule, cannot be decided. In rats, prolonged administration of thiourea derivatives has caused development of nodular goiters (6).

It is noteworthy, that the uptake of I^{131} was still abnormally low after one month of abstinence from the goitrogenous vegetables, at a time when the goiter had regressed. Subsequently the uptake of I^{131} rose from 4–8 per cent to 19 per cent. This rise may be even more significant because it was associated with a decrease in the size of the gland. In spite of the significantly low uptake of I^{131} (4–8 per cent) there were no findings indicative of severe hypothyroidism; the BMR was never lower than −15 per cent, the serum cholesterol level was only moderately increased, the EKG was normal, the skin was moist and of normal texture, and the subjective complaints of tiredness, forgetfulness and drowsiness appeared to be very mild. No explanation for this discrepancy is apparent at this time.

SUMMARY

A case of goiter in a woman habitually ingesting large amounts of rutabaga, cabbage and turnips is reported. The goiter regressed after she discontinued partaking of these foods. The case is presented as an incidence of struma cibaria.

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The Endocrine Society

THE 1953 ANNUAL MEETING

The Thirty-fifth Annual Meeting of The Endocrine Society will be held in the Hotel Statler, New York, N. Y., on Thursday, Friday, and Saturday, May 28, 29 and 30, 1953.

The Committee on Local Arrangements is Dr. Earl T. Engle, Chairman, with Drs. Rulon W. Rawson and Sidney C. Werner as members of the Committee.

All Scientific Sessions will be held in the Hotel Statler. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 29, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Reservations should be made directly with the Hotel Statler, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send FOUR COPIES of the title and abstract to the Vice-President, Dr. Lawson Wilkins, Johns Hopkins Hospital, Baltimore 5, Maryland, not later than FEBRUARY 1, 1953. **It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:**

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three additional copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

RECIPIENTS OF AWARDS FOR 1952

CIBA AWARD

The recipient of the Ciba Award for 1952 is *Dr. Seymour Lieberman*, Assistant Professor of Biological Chemistry at Columbia University.

Dr. Lieberman was born in New York City in 1916. He received the B.S. degree from Brooklyn College in 1936, and after further training in Chemistry at the University of Illinois, proceeded to Stanford University where he received the Ph.D. degree in 1941. It is perhaps unnecessary to say that his dissertation was on the chemistry of certain steroids. After further work with Dr. Fieser at Harvard, he spent a year in Switzerland at the laboratory of Professor Ruzicka.

The important and far-reaching studies on steroid metabolism in man that have been carried out in recent years at the Memorial Hospital and Sloan-Kettering Institute in New York City have indicated that a large number of steroids are excreted in the urine. These studies initiated by Dr. Konrad Dobriner, whose premature death is a great loss to science, have required not only painstaking methods of isolation but also the application of the most advanced technics of organic chemistry for the identification of the compounds present in this complex mixture of steroids. Indeed the solution of the problems of steroid metabolism cannot be effected without the aid of the organic chemist.

Dr. Lieberman's thirty odd papers are not only satisfying to the endocrinologist but give equal pleasure to the chemist. The classic methods of organic chemistry, analysis and synthesis, as well as the more recently introduced procedures have been used to identify with exactitude the steroid compounds isolated from the urine. Consequently, the work, whatever may be the future interpretation of the data, will remain as a classic example of the importance of chemistry to our understanding of biologic processes.

The Committee is happy to present the Ciba Award to Dr. Lieberman, a man who has demonstrated remarkable abilities in the exacting discipline of steroid endocrinology.

SQUIBB AWARD

The recipient of the 1952 Squibb Award is *Dr. J. H. Means*, Jackson Professor of Clinical Medicine (emeritus) of Harvard. Dr. Means has had a distinguished career as a scientist, educator, clinician, and administrator. He is representative of the qualified men who have made endocrinology a science. By his pioneer work in the physiology, pathology, and metabolic functions of the thyroid gland, he has been instrumental in our understanding of this gland and its relationship to other endocrine organs.

Dr. Means was born in Dorchester, Massachusetts, on June 24, 1885. He graduated from Harvard College with an A.B. degree in 1907 and from Harvard Medical School in 1911. He served a medical internship at the Massachusetts General Hospital from 1911–1913 and as Henry P. Walcott Fellow of Medicine from 1913–1916. During this fellowship, he studied with Professors August Krogh in Copenhagen and Joseph Barcroft in Cambridge, England. Following two years of service in the Army Medical Corps, he returned to the M.G.H. to continue his investigations on basal metabolism and the thyroid gland.

Dr. Means was appointed Jackson Professor of Clinical Medicine at Harvard Medical School and Chief of the Medical Service at the M.G.H. in 1923, in which capacities he ably served 28 years until retirement age was reached. During this period, he conducted with distinction the administrative and teaching duties and important research which resulted in a bibliography of about 200 titles, including two books. His contributions run the gamut of our knowledge of the thyroid, from basal metabolic studies, through antithyroid and goitrogenic drugs, to control of the benign and malignant diseases of the thyroid by x-irradiation and the clinical use of radioactive iodine.

His extra-curricular activities include membership in many learned societies, in some of which he has served as president or in other official capacities, meanwhile being honored with several visiting professorships and lectureships. To his most recent appointment as Staff Physician to the Medical Department of the Massachusetts Institute of Technology, Dr. Means has brought his wealth of experience, technical skill, and knowledge, which both stimulate and temper his work and that of his associates. The members of the Committee are happy to present the Squibb Award for 1952 to Dr. James Howard Means.

AYERST, McKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship for 1952 is awarded to *Dr. John Coleman Laidlaw.*

Dr. Laidlaw, a native of Canada, received the B.A., M.A., and M.D. degrees from the University of Toronto in the years from 1942 to 1947. Following a period of service in the Royal Canadian Navy, he worked at the University of London under the direction of Professor F. G. Young and received the Ph.D. degree in Biochemistry from that University in 1950.

During the past year, he has been a Research Fellow in Medicine at the Peter Bent Brigham Hospital in Boston. The Fellowship is awarded to him so that he may continue the studies he has already begun there on the effect of adrenal cortical hormones on the amino acids of serum.

SCHERING FELLOWSHIP

The Schering Fellowship in Endocrinology for 1952 is awarded to *Dr. Claiborne L. Courtright.*

Dr. Courtright received the Bachelor of Science degree from Oklahoma A. and M. College in 1948, and the M.D. degree from the School of Medicine, University of Oklahoma, in 1951. This year he has served as an interne at St. Joseph's Mercy Hospital, Ann Arbor, Michigan.

The Fellowship is awarded to him so that he may begin a study on the influence of the parathyroid hormone on bone. This will be conducted at the Oklahoma Medical Research Institute, under the direction of Dr. E. C. Reifenstein, Jr.

1953 AWARDS AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1953 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1954.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson in 1950. Dr. John D. Stoekle was chosen as the recipient of the 1951 Fellowship. Inasmuch as he was unable to accept, due to a call to military service, the same Fellowship was reawarded in 1952 to Dr. C. L. Courtright. The 1953 Schering Fellowship will be in the amount of $5,000 and will henceforth be given in the same amount on alternate years.

Awards

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH AWARD

This Award of $1,000 was established in 1939 by The Squibb Institute for Medical Research and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. None was given in 1943. This Award was increased to the amount of $2,500 beginning with the 1952 Award and the recipient was Dr. James H. Means. A special committee of five members of the Society selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1953*.

FIFTH ANNUAL POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

Sponsored by

THE ENDOCRINE SOCIETY

Roney Plaza Hotel

Miami Beach, Florida *November 3–8, 1952*

The faculty will consist of 20 clinicians and investigators eminently qualified in the fields of endocrinology and metabolism.

The program will consist of lectures, clinics, laboratory demonstrations and scientific exhibits.

The 64 lectures to be presented will cover all phases of endocrinology and metabolic disorders. Ample time will be given for discussions and answers at the end of each session and registrants are encouraged to contact members of the faculty for individual discussions. The entire program has been designed to interest not only the specialist but those in general practice and has been approved by the American Academy of General Practice for postgraduate credit.

A simultaneous translation of all lectures into Spanish will be provided through earphones.

A copy of the proceedings of the Assembly will be available to all registrants.

The Roney Plaza with its tropical gardens, outdoor swimming pool, putting greens, private beach and Cabana Club, has long been recognized as one of Florida's great beach hotels and offers special convention rates to members of this Assembly. The Surf Room, in which the sessions will be held, is delightfully air conditioned and has subdued lighting which permits taking of notes and viewing lantern slides with comfort.

A fee of $75 will be charged for the entire course and the attendance will be limited to 200. *Registration will be in the order of checks received and will close on October 3, 1952.* Should there be an insufficient number of applicants to fill the course, the registration fee will be refunded immediately in its full amount.

Please forward application on your letterhead, together with check payable to The Endocrine Society, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 N. Walker Street, Oklahoma City 3, before October 3, 1952. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Roney Plaza Hotel, Miami Beach, Florida, and the hotel advised that you are attending this Postgraduate Assembly.

PROGRAM

Monday, November 3, 1952

MORNING SESSION

8:00– 9:00 Registration

GENERAL PRINCIPLES AND PROCEDURES

9:00– 9:45 General Principles of Endocrinology—Dr. Starr
9:45–10:30 General Interrelationships of Endocrine Glands—Dr. Forsham
10:30–10:45 Neuro-Hypophyseal Relationships—Dr. Lloyd
10:45–11:00 Intermission—Refreshments
11:00–12:00 Endocrine Control of Metabolism—Dr. Engel
12:00–12:45 Diagnostic Approach to Endocrine Problems; Evaluation of Laboratory Aids in Diagnosis—Dr. Escamilla

AFTERNOON SESSION

2:00– 2:30 Abuse of Hormone Therapy—Dr. Rynearson
2:30– 3:00 Question and Answer Period—Dr. Starr, Moderator

ANTERIOR PITUITARY

3:00– 3:15 Chemistry and Physiology of the Anterior Pituitary—Dr. Klinefelter
3:15– 4:00 Anterior Pituitary Hypofunction Syndromes: Panhypopituitarism, Simmond's Cachexia, Sheehan's Syndrome, Anorexia Nervosa, and Fröhlich's Syndrome—Dr. Escamilla
4:00– 4:15 Intermission—Refreshments
4:15– 4:45 Anterior Pituitary Hyperfunction Syndromes: Gigantism and Acromegaly —Dr. Schneider
4:45– 5:00 Other Anterior Pituitary Syndromes: Pituitary Tumors—Dr. Klinefelter
5:00– 5:30 Question and Answer Period—Dr. Escamilla, Moderator
6:30 GET-ACQUAINTED PARTY—Cocktails and Dinner

Tuesday, November 4, 1952

ADRENAL CORTEX

MORNING SESSION

9:00– 9:30 Chemistry and Physiology of Adrenal Cortex—Dr. Engel
9:30–10:00 General Adaptation Syndrome; Diseases of Adaptation—Dr. Browne
10:00–11:00 Adrenal Cortical Hypofunction Syndromes: Addison's Disease and Adrenal Cortical Insufficiency of Pituitary Origin—Dr. Forsham
11:00–11:15 Intermission—Refreshments
11:15–12:00 Adrenal Cortical Hyperfunction Syndromes: A. Cushing's Syndrome and Disease—Dr. Browne
12:00–12:45 Adrenal Cortical Hyperfunction Syndromes: B. Adrenogenital and Related Syndromes—Dr. Wilkins

AFTERNOON SESSION

2:00– 2:45 ACTH and Cortisone in Non-Endocrine Disorders: Arthritis, Collagen Diseases, and so forth—Dr. Browne

ADRENAL MEDULLA

2:45– 3:15 Chemistry and Physiology of Adrenal Medulla; Adrenal Medullary Syndromes: Pheochromocytoma—Dr. Schneider

3:15– 3:45 Adrenals and Non-Endocrine Disorders: Adrenals and Hypertension; Adrenalectomy as Therapy—Dr. Forsham

3:45– 4:00 Intermission—Refreshments

4:00– 4:30 Question and Answer Period—Dr. Forsham, Moderator

4:30– 5:30 Case Presentations—Pituitary and Adrenal Disorders

FREE EVENING

Wednesday, November 5, 1952

MORNING SESSION

PANCREAS

9:00– 9:30 Chemistry and Physiology of Pancreas; Physiologic Action of Insulin; Lipocaic—Dr. Lamar

9:30–10:00 Pancreas Hypofunction Syndrome: Diabetes Mellitus: A. Etiology, Symptoms and Signs, Laboratory Findings and Diagnosis, Prognosis—Dr. Escamilla

10:00–10:30 Pancreas Hypofunction Syndrome: Diabetes Mellitus: B. Treatment of Acute State: Diabetic Acidosis—Dr. Schneider

10:30–11:00 Pancreas Hypofunction Syndrome: Diabetes Mellitus: C. Treatment of Chronic State: Diet and Insulin—Dr. Lamar

11:00–11:15 Intermission—Refreshments

11:15–11:30 Pancreas Hypofunction Syndrome: Diabetes Mellitus: D. Insulin Problems: Reaction or Coma and Treatment, Sensitivity, Resistance, Allergy, Lipodystrophy—Dr. Rynearson

11:30–12:15 Pancreas Hypofunction Syndrome: Diabetes Mellitus: E. Complications and Special Situations: Vascular Complications, Retinopathy, Neuropathy, Nephropathy; Childhood, Pregnancy, Surgery, Infections, and Old Age—Dr. Schneider

12:15–12:45 Pancreas Hypofunction Syndrome: Diabetes Mellitus: F. Diabetes Complicating Other Endocrine Disorders—Dr. Rynearson

12:45– 1:15 Pancreas Hyperfunction Syndrome; Pancreatic Islet Tumors; Functional Hypoglycemia—Dr. Rynearson

FREE AFTERNOON

EVENING SESSION

7:30– 7:45 Insulin in Non-Endocrine Disorders—Dr. Escamilla

7:45– 8:00 Non-Pancreatic Endocrine Factors in Carbohydrate Metabolism—Dr. Engel

8:00– 8:30 Question and Answer Period—Dr. Lamar, Moderator

NUTRITION

8:30– 8:45 Interrelationships of Hormones, Vitamins and Nutrition—Dr. Lamar

8:45– 9:15 Obesity and Thinness—Dr. Rynearson

POSTERIOR PITUITARY

9:15–10:00 Chemistry, Physiology, and Neurohypophyseal Interrelationships of the Posterior Pituitary; Posterior Pituitary Syndromes—Dr. Lloyd

Thursday, November 6, 1952

THYROID

MORNING SESSION

9:00– 9:30	Chemistry and Physiology of Thyroid—Dr. Rawson
9:30–10:15	Thyroid Hypofunction Syndromes: Hypothyroidism and Myxedema—Dr. Starr
10:15–11:00	Thyroid Hyperfunction Syndromes: Hyperthyroidism, Thyrotoxicosis and Graves' Disease—Dr. Rawson
11:00–11:15	Intermission—Refreshments
11:15–12:00	Other Thyroid Syndromes: Non-Toxic Goiter; Cancer; Thyroiditis—Dr. Rawson
12:00–12:15	Thyroid Hormone in Non-Endocrine Disorders—Dr. Starr
12:15–12:45	Question and Answer Period—Dr. Rawson, Moderator

ADOLESCENCE AND CHILDHOOD

AFTERNOON SESSION

2:00– 2:30	Physiology of Adolescence; Constitutional Variations in the Pattern; Precocious and Delayed Puberty; Prematurity—Dr. Wilkins
2:30– 3:15	Endocrine and Other Factors Influencing Somatic Growth and Development; Endocrine Syndromes in Childhood: A. Dwarfism—Dr. Wilkins
3:15– 3:30	Endocrine Syndromes in Childhood: B. Hypothyroidism—Dr. Wilkins
3:30– 4:00	Question and Answer Period—Dr. Wilkins, Moderator
4:00– 4:15	Intermission—Refreshments

MISCELLANEOUS

AFTERNOON SESSION

4:15– 4:30	Pseudoendocrinopathies—Dr. Turner
4:30– 5:30	Case Presentations—Pancreatic and Thyroid Disorders

FREE EVENING

Friday, November 7, 1952

OVARIES

MORNING SESSION

9:00– 9:30	Chemistry and Physiology of Ovaries; Physiology of Menstruation—Dr. Lloyd
9:30–10:00	Ovarian Hypofunction Syndromes: A. Hypergonadotropic Syndromes: Castrates; Menopause; Hypogonadism—Dr. Brown
10:00–10:15	Ovarian Hypofunction Syndromes: A. Hypergonadotropic Syndromes: Ovarian Agenesis and Turner's Syndrome—Dr. Turner
10:15–11:00	Ovarian Hypofunction Syndromes: B. Hypogonadotropic Syndromes: Hypogonadism, Ovarian Failure Secondary to Pituitary Failure—Dr. Greenblatt
11:00–11:15	Intermission—Refreshments
11:15–11:45	Ovarian Hypofunction Syndromes: C. Normogonadotropic Syndromes: Metropathia Hemorrhagica; Dysmenorrhea, Endometriosis—Dr. Greenblatt
11:45–12:00	Ovarian Hypofunction Syndromes: C. Normogonadotropic Syndromes: Hypothalamic Amenorrhea—Dr. Reifenstein
12:00–12:15	Ovarian Hyperfunction Syndromes and Tumors: Granulosa Cell, Theca Cell, Luteoma, and Others—Dr. Greenblatt
12:15–12:45	Endocrine Aspects of Benign Breast Disorders in the Female—Dr. Nathanson
12:45– 1:15	Question and Answer Period—Dr. Turner, Moderator

FREE AFTERNOON

PLACENTA

EVENING SESSION

7:30– 7:45	Chemistry and Physiology of the Placenta—Dr. Brown
7:45– 8:30	Physiology of Pregnancy; Endocrine Aspects of Dysfunctions in Pregnancy; Habitual Abortion, Toxemias of Pregnancy, Pregnancy in Diabetes, Chorionepithelioma, Hydatidiform Mole, and so forth—Dr. Brown
8:30– 9:00	Sterility in the Female—Dr. Simmons
9:00– 9:30	Gonadal Hormones in Non-Endocrine Disorders in Females: Cancer of the Breast and Other Organs, Convalescence—Dr. Nathanson
9:30–10:00	Question and Answer Period—Dr. Brown, Moderator

Saturday, November 8, 1952

TESTES

MORNING SESSION

9:00– 9:15	Chemistry and Physiology of Testes—Dr. Heller
9:15– 9:45	Testicular Hypofunction Syndromes: A. Hypergonadotropic Syndromes: Eunuchs, Eunuchoids, Male Climacteric—Dr. Heller
9:45–10:00	Testicular Hypofunction Syndromes: A. Hypergonadotropic Syndromes: Klinefelter's Syndrome—Dr. Klinefelter
10:00–10:30	Testicular Hypofunction Syndromes: B. Hypogonadotropic Syndromes: Eunuchoids, Testicular Failure Secondary to Pituitary Failure—Dr. Heller
10:30–10:45	Testicular Hyperfunction Syndromes and Tumors: Leydig Cell, Chorionepithelioma and Others—Dr. Simmons
10:45–11:15	Endocrine Aspects of Breast Disorders in the Male: Gynecomastia; Adolescent Mammoplasia—Dr. Klinefelter
11:15–11:30	Intermission—Refreshments
11:30–12:00	Sterility in the Male—Dr. Simmons
12:00–12:30	Gonadal Hormones in Non-Endocrine Disorders in Males: Cancer of the Prostate and Other Organs, Convalescence—Dr. Nathanson
12:30–12:45	Cryptorchidism—Dr. Heller

AFTERNOON SESSION

2:00– 2:30	Question and Answer Period—Dr. Klinefelter, Moderator

PARATHYROIDS

2:30– 2:45	Chemistry and Physiology of Parathyroids—Dr. Reifenstein
2:45– 3:15	Parathyroid Syndromes: Hypo- and Hyper-parathyroidism—Dr. Reifenstein
3:15– 3:45	Metabolic Bone Diseases; Relation to Endocrine Glands: Osteitis Fibrosa Generalisata, Osteomalacia, and Osteoporosis—Dr. Reifenstein
3:45– 4:00	Intermission—Refreshments
4:00– 4:30	Question and Answer Period—Dr. Reifenstein, Moderator
4:30– 5:30	Case Presentations—Gonadal and Parathyroid Disorders

FACULTY

Dr. Willis E. Brown, Professor and Head of Department of Obstetrics and Gynecology, University of Arkansas School of Medicine, Little Rock, Arkansas.

Dr. John S. L. Browne, Professor of Medicine, McGill University; Director, McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Dr. Frank L. Engel, Associate Professor of Medicine and Associate in Physiology, Duke University School of Medicine, Durham, N. C.

Dr. Roberto F. Escamilla, Associate Clinical Professor of Medicine, University of California School of Medicine, San Francisco, California.

Dr. Peter H. Forsham, Associate Professor of Medicine and Pediatrics, University of California School of Medicine, San Francisco, California.

Dr. Robert B. Greenblatt, Professor of Endocrinology, Medical College of Georgia, Augusta, Georgia.

Dr. Carl G. Heller, Associate Clinical Professor of Medicine, University of Oregon, Medical School, Portland, Oregon.

Dr. Harry F. Klinefelter, Jr., Instructor in Medicine, Johns Hopkins Medical School, Baltimore, Maryland.

Dr. Carlos P. Lamar, Director, Department of Endocrinology and Metabolism, Jacks Memorial Hospital, Miami, Florida.

Dr. Charles W. Lloyd, Departments of Medicine and Obstetrics, State University of New York, Medical Center of Syracuse, Syracuse, N. Y.

Dr. Ira T. Nathanson, Associate Clinical Professor of Surgery, Harvard Medical School; Associate Visiting Surgeon, Massachusetts General Hospital, Boston, Massachusetts.

Dr. Rulon W. Rawson, Attending Physician, Memorial Center; Chief, Division of Clinical Investigation, Sloan-Kettering Institute; and Professor of Medicine, Sloan-Kettering Division of Cornell University Medical College, New York, N. Y.

Dr. Edward C. Reifenstein, Jr., Director, Oklahoma Medical Research Institute and Hospital; Professor of Research Medicine, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

Dr. Edward H. Rynearson, Head, Section of Endocrinology and Metabolism, Mayo Clinic, Rochester, Minnesota.

Dr. Robert W. Schneider, Member, Department of Endocrinology and Metabolism, Cleveland Clinic; Associate Professor of Internal Medicine, Frank E. Bunts Educational Institute, Cleveland, Ohio.

Dr. Fred A. Simmons, Instructor in Gynecology, Harvard Medicine School; Assistant Surgeon, Massachusetts General Hospital, Boston, Massachusetts.

Dr. Paul Starr, Professor and Chairman, Department of Internal Medicine, University of Southern California School of Medicine, Los Angeles, California.

Dr. Henry H. Turner, Clinical Professor of Medicine, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

Dr. Lawson Wilkins, Associate Professor of Pediatrics, Johns Hopkins Hospital, Baltimore, Maryland.

The American Goiter Association

1953 ANNUAL MEETING

The 1953 Annual Meeting will be held *May 7, 8 and 9, 1953*, in the Drake Hotel, Chicago, Illinois.

VAN METER PRIZE AWARD FOR 1953

The American Goiter Association again offers the Van Meter Prize Award of Three Hundred Dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association, which will be held in Chicago, Illinois, May 7, 8 and 9, 1953, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, not later than *February 15, 1953*. The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association.

THE NEW YORK ACADEMY OF MEDICINE
THE TWENTY-FIFTH GRADUATE FORTNIGHT

October 6–17, 1952

HORMONES IN HEALTH AND DISEASE

21 Evening Lectures—4 Morning Panel Discussions—20 Afternoon Hospital Clinics—Scientific Exhibits

Evening Lectures

Speakers

Fuller Albright	E. C. Kendall	De Witt Stetten, Jr.
David P. Barr	Joseph B. Kirsner	Cyrus C. Sturgis
D. M. Bergenstal	Abbie I. Knowlton	Howard C. Taylor, Jr.
Francis J. Braceland	Charles Ragan	H. B. van Dyke
George F. Cahill	Rulon W. Rawson	Sidney C. Werner
Frank L. Engel	I. Snapper	Abraham White
Joseph W. Jailer	Louis J. Soffer	Lawson Wilkins

Morning Panels (Wednesday & Friday, Each Week)

Chairmen

David P. Barr; Louis Leiter; Rustin McIntosh; C. P. Rhoads

Participating Hospitals

Bellevue	Montefiore	Presbyterian
Beth Israel	Morrisania	Roosevelt
Flower-Fifth Avenue	Mount Sinai	St. Luke's
Harlem	New York	University (Post-Graduate)
Memorial	Polyclinic	Veterans Administration

REGISTRATION FEE: $10.00 for the Entire Program—$5.00 for Second Week.

Medical Officers of the Armed Services, in uniform, will be admitted without charge.

Interns and Residents will be admitted without charge, provided they present letters from their chiefs of service. They will be seated as facilities permit, after Fellows of the Academy and other registrants have been accommodated.

A program will be mailed to every Fellow of the Academy without request and to other physicians upon request. *Address request to Secretary, Graduate Fortnight, 2 East 103 Street, New York 29, N. Y.*

BOOKS RECEIVED

Diabetes and Pregnancy. Blood Sugar of Newborn Infants During Fasting and Glucose Administration. By JØRGEN PEDERSEN, Lying-in Department B, Rigshospitalet, Copenhagen; 230 pages; 23 figures; 44 tables; 188 references; 1952. Danish Science Press, Ltd., Copenhagen, Denmark.

Die Osteomalazie. By Dr. Med. MARKUS WERNLY, Bern; 101 pages; 16 illustrations; extensive references; 1952. Grune & Stratton, New York, agents for Georg Thieme Verlag, Stuttgart. Price, paper, DM 14.40.

Endocrine Diagnosis. By H. UCKO, M.D., London, England; 529 pages; 84 figures; 26 tables; 1951. Staples Press, London and New York.

Endocrinologie, clinique, thérapeutique et expérimentale. By P. SAINTON, H. SIMONNET and L. BROUHA. Third edition, in 2 volumes, entirely revised by P. SAINTON and H. SIMONNET; 1518 pages (18×25); 249 figures; 1952. Masson et Cie, Paris. Brochés, 7,500 fr.; cartonnés toile, 8,800 fr.

Functional Endocrinology From Birth Through Adolescence. By NATHAN B. TALBOT, M.D. Associate Professor of Pediatrics, Harvard University and Physician, Children's Medical Service, Massachusetts General Hospital, Boston; EDNA H. SOBEL, M.D., Instructor in Pediatrics, University of Cincinnati College of Medicine and Research Associate, Children's Hospital Research Foundation and the Fels Research Institute, Antioch College; JANET W. MCARTHUR, M.D., Instructor in Gynecology, Harvard University and Asst. Physician, Children's Medical Service, Massachusetts General Hospital; and JOHN D. CRAWFORD, M.D., Instructor in Pediatrics, Harvard University and Asst. Physician, Children's Medical Service, Massachusetts General Hospital; 668 pages; numerous tables and figures; extensive bibliography; 1952. Published for the Commonwealth Fund by Harvard University Press, Cambridge, Massachusetts. Distributed in Great Britain by Geoffrey Cumberlege, Oxford University Press, London. Price $10.00.

Roentgenology in Obstetrics and Gynecology. Ed. 2. By WILLIAM SNOW, M.D., Chief Radiologist, Veterans Hospital; Consulting Radiologist, Willis-Knighton Memorial Hospital and Clinic, Shreveport, La.; 376 pages; 273 figures; 1952. Charles C Thomas, Publisher, Springfield, Ill. Price $10.50.

Studies on Testis and Ovary, Eggs and Sperm. Edited by EARL T. ENGLE, Ph.D., College of Physicians and Surgeons, Columbia University, New York, Chairman of the Conference for the Committee on Human Reproduction, National Research Council; a symposium by 17 contributors; 244 pages; numerous figures and tables; 1952. Charles C Thomas, Publisher, Springfield, Illinois. Price $7.50.

The Thyroid. By THOMAS HODGE MCGAVACK, M.D., Professor of Clinical Medicine, New York Medical College; Director of the New York Medical College, Metropolitan Hospital Research Unit—with a section on surgery by JAMES M. WINFIELD, M.D., Professor and Director of Surgery, New York Medical College, and WALTER L. MERSHEIMER, M.D., Associate Professor of Surgery, New York Medical College —and a section on history by DOROTHY B. SPEAR, Ph.D., Librarian, and THOMAS HODGE MCGAVACK, M.D.; 646 pages; 22 tables; 72 figures; extensive bibliography; 1951. The C. V. Mosby Company, St. Louis, Mo. Price $13.50.